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A New mite, *Zwickia gibsoni* n.sp., Fam. Anoetidae, from the pitchers of *Sarracenia purpurea* L.

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When in 1948 I first made the acquaintance of the mite about to be described I was intrigued with the idea that a form morphologically so similar to those described by Oudemans, 1915 and Hirst 1928 should come from such a different habitat. Whilst recognizing the totally different geographical areas, sphagnum bogs of boreal Canada as opposed to humid forests of tropical Ceylon and Malaya, the question arises as to whether the micro-habitat in which these three species live is so totally different. From the published material available (vide Thienemann 1928-29), which I admit is not very extensive, there does not seem to be much chemical difference between the waters of *Nepenthes* and those of *Sarracenia.* The similarity of these two micro-habitats is further substantiated by the fact that copepods of the genus *Parastenocaris* have been found in the pitchers of *Sarracenia* which closely resemble those described by Menzel 1921, Chappius 1931 and Thienemann 1928-29 (quoting from Ghosh, no reference given) from the water of *Nepenthes.* The question of distribution over such great distances and over what must be long ages poses certain difficulties which can only be answered when we know more about the past history and distribution of pitcher plants and mites. The possession of an hypopial stage by all three species does offer some help in the problem but the further question of how the Canadian mites have adapted themselves to the rigours of a northern winter is difficult to understand.

Description: *Female* (vide fig. 1) Exclusive of the gnathosoma the females of this species measure, on the average, .43 mm. in length. As in all known Anoetids the subchelate chelicerae are serrate, each being provided with approximately 22 minute teeth which decrease in size towards the tip of this member. On the ventral surface the first joint of the two-jointed pedipalp bears distally a small seta, the second, or wrinkled joint is provided with two seta, one directed mesially the other anteriorly.

The propodosoma carries dorsally four pairs of setae of varying lengths*; the cervical (.4 x 4c); the inner propodosomatic (1 x 4c); the outer propodosomatic (3 x 4c); and a pair of structures which Oudemans (1924) states are the nuchal (= Grandjean's organ △) setae rather than the rostral (= vertical Oudemans' terminology) as had previously been maintained. These structures are unlike the nuchal setae of acarids in that they do not arise from the lateral face of the propodosoma and are unlike true propodosomatic setae in that they are displaced laterally and so close to the margin of the propodosoma that they appear to be processes of the latter rather than distinct setae. Ventrally there is only one fairly long (1 x 4c) anterior interepimeral bristle between the first and second epimera. The opisthosoma bears dorsally eleven pairs of setae: the inner

*See Nesbitt, 1945 for the terminology employed in naming these setae. It should be mentioned that the inner humeral seta (No. 4c) which is approximately equal in length to genu I has been employed as the unit of measurement.

(No. 4c), middle (3 x 4c) and outer (3.3 x 4c) humerals; the first (No. 5=.8 x 4c), second (No. 6=2.6 x 4c), and third (No. 7=2. x 4c) lumbars; the outer (No. 8=3.6 x 4c), middle (No. 10=3 x 4c) and inner (No. 9=2.8 x 4c) submarginals; and the marginal (No. 14=3.4 x 4c) setae. In addition to these setae which this species has in common with the Acarids there is another seta (6b=3.2 x 4c) which is regarded as being related to seta No. 6 in that it arises just anterad of this seta and the execretory pore. Ventrally the opisthosoma bears one pair of posterior interepimeral bristles, two pairs of what might be considered paragenital bristles (these arise close to and slightly mesiad of the ring organs), three pairs of minute anal bristles, and one pair of larger (No. 13=2.8 x 4c) posterior postanal setae. In addition to the setae already described the dorsal surface bears a propodosomatic shield which is mildly shagreened, a pore-like marking between the humeral and second lumbar setae, the excretory pore anterad of the outer submarginal seta, a marking reminiscent of a seta pit on the margin of the body just anterad of the marginal setae, and the opening, of the bursa copulatrix. The opening of the vulva is bounded anteriorly, by a yoke-shaped bar which curves abruptly about the anterior ring organs, and posteriorly by a similar bar in the form of a flat V. Just posterad of the hindmost margin of the vulva and slightly out from the mid line is a small pore-like marking.

As the pretarsal segments of all four legs have the usual complement of setae, and as they are adequately figured (vide fig. 1), there is little need to comment upon them here. The first tarsus is somewhat reminiscent of a generalized rhizoglyphid in that the macro and micro-sense setae appear to arise for the same seta pit, in that these latter are preceded distally by a small blunt spine and in that there are four distinct setae in a whorl about the middle of the tarsus; it differs, however, in lacking any trace of parasub-basal or sub-basal setae. Terminally tarsus I bears a long sickle-shaped claw, two setae one of which is twice as long, the other, about one-half as long as the claw, two dorsal and three ventral spines. There appears to be no trace of a caruncle on any of the tarsi. Tarsus II bears a macrosense seta proximally, four setae in the middle of the segment, the dorsal of which is slightly lanceolate in shape, two dorsal and three ventral distal spines, two terminal setae neither of which is as long as the claw, and the claw. As the terminal setae on tarsi III and IV are only about three-quarters as long as the claw they are noticeably shorter than the similar structures on the first two tarsi.

Male (fig. 3) Exclusive of the gnathosoma the males of this species measure on the average .3 mm. in length. Unlike that of the female, the palp of the male appears to have only one distal seta. The mesially directed member is wanting. The chaetotactic pattern which may be expressed as follows is similar to that of the female except that as the drawing shows, the setae are absolutely and relatively somewhat longer: seta 3a=4c*; 3b=2.2 x 4c; 4a=2 x 4c; 5=.4 x 4c; 6a and 6b=1.9 x 4c; 7=1.2 x 4c; 8=1.8 x 4c; 9=1.5 x 4c; 10=1.7 x 4c; 13=1.7 x 4c; 14=2 x 4c. On the ventral surface the anterior and posterior interepimeral, and the "paragenital", bristles are in relatively the same position and have much the same length as in the female. A somewhat longer bristle arises from the genital operculum laterad of the base of the aedeagus and the anterior commissure of the anus. As in the female the epimera are heavily sclerotized and readily noticeable. The aedeagus which is covered by its divided genital cover or operculum, is heavily sclerotized and bears a blunt recurved tip.

The legs differ from those of the female in being relatively longer and heavier. The setae pattern is fundamentally the same except that in the males the

*It should be noted that in the male seta 4c is longer than genu I by about one-third of its length.

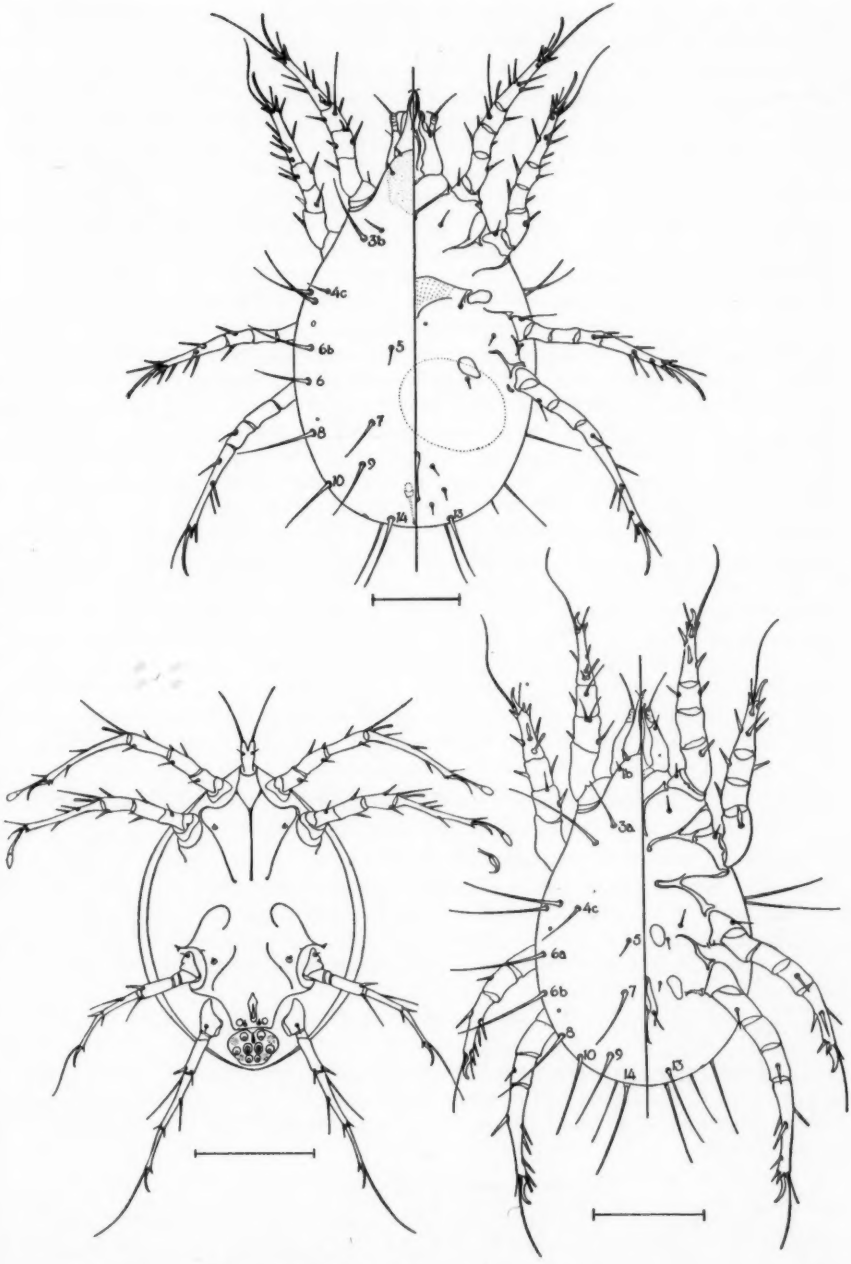

Fig. 1. View of the female, dorsal side left, ventral side right.
Fig. 2. View of the hypopus.
Fig. 3. View of the male, dorsal side left, ventral side right.
Note: on all three drawings the heavy line beneath the drawing represents .1 mm.

claws are much shorter and heavier, and the terminal setae on tarsi III and IV are at least twice as long as the claw.

Hypopus. (vide fig. 2). The hypopi of this species measure on the average .25 mm. in length and slightly less than .2 mm. in width. They are thus broad oval in shape, flat on the ventral surface and slightly but evenly domed dorsally. The dorsal surface appears to be free of markings of any kind and is provided with a few minute setae which seem to bear little or no relationship to the chaetotactic pattern of the adult. Unfortunately, I have not as yet found any nymphs or larvae hence I cannot compare their chaetotactic pattern with either that of the hypopus or the adults. On the ventral surface the anterior and posterior interepimeral processes are represented in this species by minute peg-like structures; there is only one pair of para-anal bristles and each of these is flanked laterally by a small sucker or ring-shaped organ. The gnathosoma is bifurcate distally and extends well beyond the anterior margin of the body (notocephalic plate). It bears basally a pair of minute bristles and terminally a pair of distinct setae. The adhesive plate is one-third broader than it is long and provided with four pairs of what might be considered suckers and two pairs of circular depressed areas behind the outermost pairs of "suckers". Exclusive of the coxae and claws the four pairs of legs are slightly longer than one half of the length of the body. The anterior pairs (I and II) are terminated by a sickle-shaped claw as in the female, a spoon or leaf-shaped seta and a terminal seta which is somewhat the same length as the claw. Legs III and IV lack the modified seta but bear a terminal seta which is three times as long as the claw on leg III and four times the length of the claw on leg IV. Legs III and IV differ further from I and II in that what appears to be the tarsus is bent in III, and broken into two subsegments is IV. My reason for believing that these are subsegments of the tarsus rather than the tibia and tarsus is based on the fact that what could be considered the terminal tibial seta arises distally from the segment which precedes the member in question. This interpretation, however, implies that leg IV is lacking a segment or joint.

Type Habitat: In the pitchers of *Sarracenia purpurea* L.

Holotype: ♀, Danford Lake area, Quebec, August 25, 1953 (H. H. J. Nesbitt); No. 6134, in the Canadian National Collection, Ottawa.

Allotype: ♂, same data, on same slide as holotype.

Paratypes: on Holotype slide and under same cover glass: 7 ♀♀, 1 ♂ and 6 immature ♀♀; under a second cover glass 5 hypopi. On a different slide but same data: 2 ♀♀, 1 atypical ♂ (Leg IV right side not formed) and 9 immature ♀♀ under one cover; 5 hypopi under a separate cover glass.
1 ♂, 5 ♀♀ Aylesford, N.S., July 20, 1949 (H.H.J.N.).
2 ♀♀, 1 hypopus, Mer Bleue (near Ottawa) Ontario, April 13, 1948, (H.H.J.N.).

The specific name *gibsoni* has been given to the new species described above in honour of C. C. Gibson, Q.C., who assisted this work by placing his sphagnum bogs at Glenfarne (Danford Lake, Aylwin Township, Gatineau County, Quebec) at our disposal.

Family *Anoetidae* Oudemans 1904

In discussing the family Anoetidae, Oudemans, 1924, mentioned among other things those characteristics which he thought were peculiar to the group. These may be listed as follows: "No pseudostigmatic organs. No clasping organs. The vertical hairs are short, placed on the foremost edge and bent downwards, or they are absent by caducity . . . The ♀ genital aperture is a transverse slit between propodo- and hysterosoma. . . .

A.1. With line of demarcation between propodo- and hysterosoma ..... *Anoetus* Dug. 1842
A.2. Without such line Zwickia nov-gen. Type *Anoetus guentheri* Oudms. 1916
Probably hereto also *A. muscarum* L. 1758, which I have not had under observation."

Key for the separation of the known species of the genus *Zwickia* Oudms. 1924

1. With the terminal tarsal setae of legs III and IV three to four times as long as the tarsus ..... *Z. guentheri* Oudms. 1916*
With the terminal tarsal setae of legs III and IV noticeably shorter than the length of the tarsus 2
2. With the posterior marginal setae (1 pair) of both sexes greater in length than the width of the opisthosoma at the widest ..... *Z. nepenthesiana* Hirst 1928
With the posterior marginal setae at most only equal to one-half of the width of the opisthosoma ..... *Z. gibsoni* n. sp.

*For a full description and adequate figures of this species, see Vitzthum, H. Graf. 1931.

References

Hirst, S. 1928. A new Tyroglyphid mite (*Zwickia nepenthesiana*, sp. n.) from the pitchers of *Nepenthes ampullaria*. *Jour. Malayan Br. Roy. Asiatic Soc.* 6 (3): 19-22.
Thienemann, A. 1928-29. "Tropische Binnengewässer". *Archiv. fur Hydrobiologie.* 11 (suppl.): 9-13.
Oudemans, A. C. 1915. *Anoetus guentheri* nov. sp. (in Guenther, K., 1915. Die lebenden Bewohner der Kannen der Pflanze *Nepenthes destillatoria* auf Ceylon). Zeitschrift f. wissen. Insektenbiologie 11(1): 242-243.
Oudemans, A. C. 1924. Acarologische Anteekeningen LXXVI. *Entom. Ber* 6(139): 309-310.
Menzel, R. 1921. Beiträge zur Kenntnis der Mikrofauna von Niederländisch-Ostindien. I Moosbewohnende Harpacticiden. *Treubia* 2: 137-145.
Chappius, P. A. 1931. Copepoda, Harpactoida der Deutschen Limnologischen Sunda—Expedition. "Tropische Binnengewässer". *I Arch. f Hydrobiol.* 8 (Suppl.): 512-584.
Vitzthum, H. Graf. 1931. Terrestrische Acarinen der Deutschen Limnologischen Sunda—Expedition. "Tropische Binnengewässer" II. *Archiv. f Hydrobiol.* 9: 121-134.
Nesbitt, H. H. J. 1945. A Revision of the family Acaridae (Tyroglyphidae) order Acari, based on comparative morphological studies. *Can. J. Res., D,* 23: 139-188.

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Note on Ancistrocerus parietum (Linnaeus) (Hymenoptera: Vespidae)

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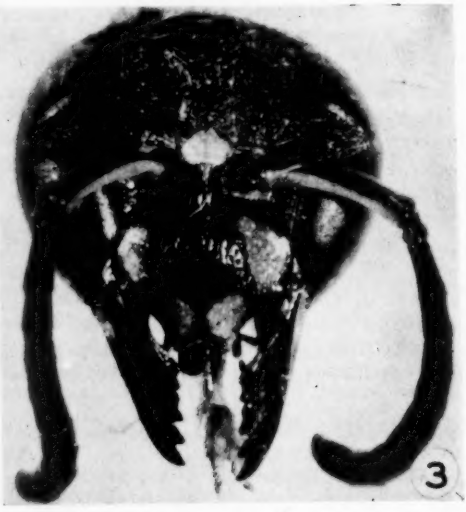
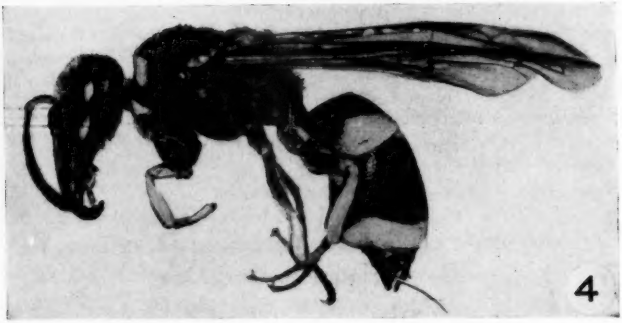
This note reports an extension in the known distribution of the species *Ancistrocerus parietum* (Linnaeus) in Canada, and a few observations on its nesting habits.

A. parietum is a European species that, according to Bequaert (1943), was recently introduced into North America, it being first recorded from Ithaca, N.Y., in 1916. Bequaert suggested that the species was gradually extending its range in North America. He noted its occurrence in the northeastern United States, as far west as Michigan and as far south as Ohio and New Jersey. He noted two Canadian records (Montreal and Kamouraska County, Quebec).

At Ottawa, the writer's attention was drawn to the species by Mr. H. L. Seamans, Field Crop Insect Unit, who first noted it on August 13, 1953, when he observed a female excavating a burrow in the mortar between two bricks outside his office window. He captured the specimen after it had completed its nest. The latter had a southwestern exposure and was approximately 40 feet above the ground.

Mr. Seamans stated that the insect excavated, provisioned, and sealed the nest in approximately three-quarters of an hour.





Figs. 1-4. *A. parietum*. 1, Nest before removal of clay plug and contents; 2, Nest after removal of clay plug and contents; 3, Anterior aspect of the head; 4, Lateral aspect.

The writer found the nest to contain five pale-green, lepidopterous larvae, each about 10 mm. long. These were determined by Dr. T. N. Freeman, Systematic Entomology Unit, as of a species of the family Tortricidae.

Bequaert (1925) gave a comprehensive description of the adult female wasp, with which the writer's specimen agrees, except for a slight variation in the colour pattern of the clypeus. In this, the transverse band at the apex is replaced by two narrowly separated spots (Fig. 3).

Fig. 1 shows the nest before and Fig. 2 after the contents were removed; Fig. 4 shows the general livery of the insect.

References

Bequaert, J. 1925. The genus *Ancistrocerus* (Hymenoptera, Vespidae) in North America with a partial key to the species. *Trans. Amer. Ent. Soc.* 51: 57-117.

Bequaert, J. 1943. The North American species of *Ancistrocerus*, proper (Hymenoptera, Vespidae). *Ent. Amer.* 23: 225-286.

(Received January 12, 1954)

Notes on the North American Species of *Aphrodes* (Homoptera: Cicadellidae)

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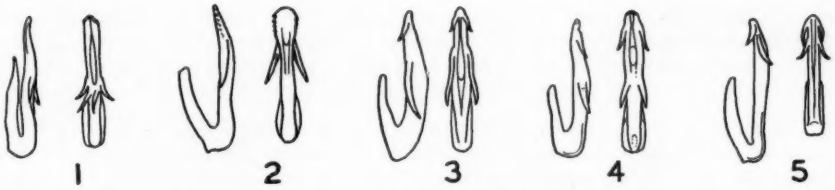
Six North American species of leafhoppers of the genus *Aphrodes* were listed, with 36 synonyms, by Oman (1949). Examination of the male genitalia has revealed not more than four species. Because of the extensive individual variation it is often difficult to identify some of the species on external characters alone. The male genitalia, however, show good specific characters (Figs. 1-5).

Aphrodes albifrons (Linn.) was first recorded from North America by Ball (1900). Examination of the male genitalia of one of Ball's specimens, among others identified on external characters as of *albifrons*, shows the species to be



Figs. 1-5. *Aphrodes* spp., lateral and ventral views of aedoeagus of: 1, *A. costata*; 2, A. *agrestis*; 3, *A. flavostrigata*; 4, *A. albifrons* (European specimen); 5, *A. fuscofasciata.*

A. flavostrigata (Don.). Because of the variation in colour and markings, *flavostrigata* may be confused with *albifrons* on external characters, particularly in the female. The true *albifrons*, of Europe, has distinct genitalic characters (Fig. 4). Though most North American records for *albifrons* probably refer to *flavostrigata*, some may refer to *mixtus* (Say), a synonym of *A. agrestis* (Fall.) that Ball (1900) and Van Duzee (1917) listed as a synonym of their *albifrons*, and a few records, based on undissected males, may refer to *A. fuscofasciata* (Goeze). *A. placida* (Prov.) was described from a single specimen, sex not stated, collected in Quebec. The species has apparently not been recognized since. The description agrees accurately with that of a common form of the female of *flavostrigata*. The indications are, therefore, that *A. albifrons* of American authors (*nec* Linnaeus) and *A. placida* (Prov.) are synonyms of *A. flavostrigata* (Don.).

Species of *Aphrodes* inhabit grasslands and are probably more common and widely distributed than the existing records indicate. They are often overlooked by collectors as they normally live on the ground beneath surface litter. In Canada. *A. costata* (Panz.) has been collected in localities in Quebec, Ontario, Saskatchewan, and British Columbia; *A. agrestis* in Quebec, Ontario, and Manitoba; *A. flavostrigata* in Nova Scotia, Prince Edward Island, Quebec, Ontario, and British Columbia (Goldstream); and *A. fuscofasciata* in Ontario (Vineland) and British Columbia (Victoria). The last two species have apparently not been recorded previously for western North America, and *A. fuscofasciata* appears to

have been recorded previously for North America only from New Jersey (Ball, 1900).

I wish to thank Dr. David A. Young, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, for the loan of specimens from the collections of the United States National Museum.

References

Ball, E. D. 1900. Notes on the Acocephalina (Homoptera: Jassidae). *Proc. Iowa Acad. Sci.* 7: 64-72.

Oman, P. W. 1949. The Nearctic leafhoppers. *Mem. Ent. Soc. Washington* 3.

Van Duzee, E. P. 1917. Catalogue of the Hemiptera of America North of Mexico.

(Received August 26, 1953)

Book Review

SIMULIIDAE DE FRANCE ET D'AFRIQUE DU NORD (Systématique, Biologie, Importance Médicale) par P. Grenier. Préface par E. Roubaud. Encyclopédie Entomologique Series A, XXIX. 171 pages, 268 figures, 2500 fr. 1953. Paul Lechevalier, editeur. 12, Rue de Tournon. Paris.

The family Simuliidae has been attracting increasing attention on a world-wide scale. There has been a need for an up-to-date systematic study of the black flies of continental Europe. Dr. Grenier's paper is the first systematic treatment of the French simuliids since the notes of E. Roubaud, 1905 to 1915 and the work of E. Séguy in 1925.

In the first part of this paper, "Généralities", he presents an up-to-date and complete summary of the external morphology and the development of the various stages of black flies, their general biology, their economic importance and pathogenic role, their natural enemies and methods of control. This is a fine supplement to his large and valuable work on the microscopic anatomy of the larvae and their ecology*.

The second and main portion of the paper treats fully each of the species found in France with good keys to larvae, pupae, and male and female adults. The keys are amply illustrated with excellent original drawings including characters not usually used systematically such as the female mandible, the buccopharyngeal or cibarial armature and many larval characters. There is a full description of the external features of each species and a discussion of the habitat in which each species is found, its distribution in France and its habits.

There has been a need for a similar systematic treatment of the North African simuliid fauna. In the third section of the paper Dr. Grenier reports on the species found in Algeria, Tunisia and Morocco in as complete a manner as the present state of knowledge permits.

This paper is invaluable to those studying the black flies of Europe and North Africa and is of considerable interest to those studying North American black flies because of the up-to-date discussion of the biology and because several of the French species treated in the systematic section are also found in North America.

DOUGLAS M. DAVIES

*P. Grenier—Contribution a l'étude biologique des simuliides de France. Physiologia Comparata et Oecologia. Vol. 1(3/4). 1949.

Persistence of Radio-Activity in Grasshoppers (Acrididae) Tagged with Phosphorus-32[1]

By R. A. FULLER[2], P. W. RIEGERT[3], AND J. W. T. SPINKS[4]

With the increased availability of radioisotopes and their application to the study of insect behaviour and dispersal, the possibility of using a radio-active tag on grasshoppers was investigated. Preliminary studies on the distribution of radio-active phosphorus (P^{32}) in *Melanoplus mexicanus mexicanus* (Sauss.) and *Cammula pellucida* (Scudd.) were carried out by Murray (1949) and Fuller (1950). The P^{32}, in the form of phosphate ion, was placed on food plants in drop form, or the leaves were dipped in an aqueous solution containing the phosphate ion. When this was fed to the grasshoppers over 50 per cent of the activity was excreted, but the amount retained was distributed throughout the body. The highest activities were recorded for the thorax and the metathoracic legs. The radio-activity apparently had no ill effects on the grasshoppers. However, more specific data were required to determine how well the applied activity is taken up and how long it is retained.

A modified method of applying the radio-activity to food material was devised. The aqueous solution of P^{32}, in the form of phosphate ion, was sprayed on growing vegetation with a manually operated atomizer. The radio-activity remained on the leaves when the solution dried. Plants so treated were eaten readily by the grasshoppers.

Wheat seedlings growing in a one-quart jar were sprayed with 2.0 ml. of solution containing 0.1 mc. of P^{32}. After this solution had dried on the leaves, 140 first-instar nymphs of *C. pellucida* were placed in the jar and allowed to feed on the treated leaves for 24 hours. The grasshoppers were then removed, placed in clean jars, and supplied with fresh, untreated wheat seedlings. The nymphs were reared in these containers under suitable laboratory conditions until they became adults. At intervals 10 individuals were removed and immobilized, and their P^{32} content was determined. They were then returned to the rearing jars.

Counts were made with a portable survey meter[5], the probe of which was clamped in a fixed position in a counting chamber. The counting apparatus was calibrated by means of a RaD+E standard, and also by counting aliquots of P^{32} solutions of known concentration. The grasshoppers were placed below the probe at a fixed distance from it so that uniform and comparable counts could be made throughout the experiment. The counts obtained were corrected for background and for radio-active decay so that they are directly comparable.

Fig. 1 shows that the radio-active content of the grasshoppers decreased rapidly during the initial few days after treatment but soon levelled off and became constant. The loss in radio-activity was, in all probability, due to excretion and ecdysis. After the fourteenth day, no appreciable activity was detected in samples of excrement and this agrees with the levelling-off shown in the curve in Fig. 1. The radio-activity of individual grasshoppers after 28 days was sufficiently high to be readily detected with the portable Geiger counter. This indicated that the persistence of the tag was highly satisfactory during this experiment, which encompassed the major growth period of the insect.

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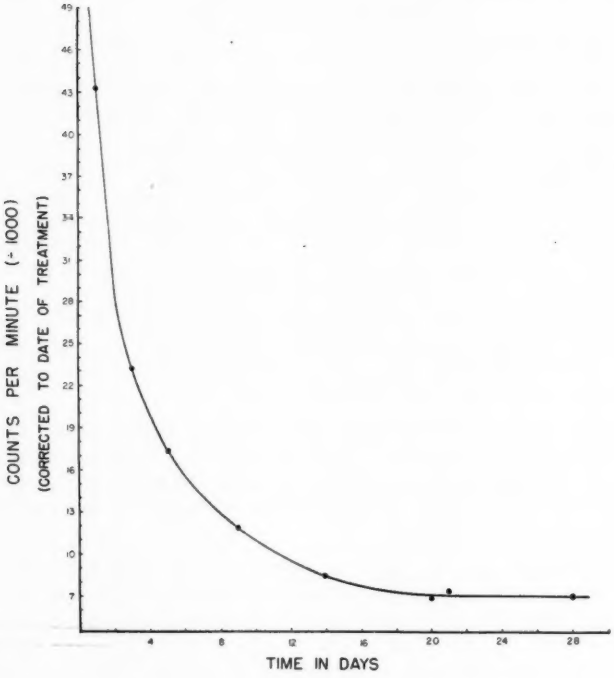

Fig. 1. Average decrease in radio-active content of single nymphs of *Cammula pellucida* (Scudd.) after treatment, by ingestion, with P^{32}.

The amount of radio-activity deposited in moulted skins was also determined. Counts were made of as many individual, intact exuviae as could be collected from the rearing jars (Table I). The high count obtained from the cast skins

TABLE I

Radio-activity of Exuviae of P32-treated nymphs of *C. pellucida*

Instar	Number counted	Disintegrations per Minute (in excess of background)		
		Maximum	Minimum	Average
First	79	6,131	198	1,683
Second	49	246	0	38
Third	22	400	0	35
Fourth	25	374	0	60
Fifth	17	147	0	25

of the first-instar nymphs may have been due to external contamination from the treated food plants. The exuviae of second-, third-, fourth-, and fifth-instar nymphs showed almost negligible radio-activity.

These data indicate that very little activity was lost through moulting. This is important in field studies where a long-persisting tag is required in an experimental animal. The principal loss, through excretion, was great at first but decreased rapidly and became unimportant after about two weeks. After 28 days, 30 of the original 140 grasshoppers were still alive and were either fifth-instar nymphs or adults. Because the survival of the P^{32}-treated grasshoppers was very nearly identical to that of laboratory-reared, normal, untreated grasshoppers, it was concluded that the radio-phosphorus had little or no deleterious effect.

The ease of treatment with P^{32} in relatively small amounts of food readily consumed by grasshoppers, the small loss of activity through moulting, and the satisfactory persistence of the radio-active tags indicate that this technique may be used in a wide range of field investigations of grasshoppers. The authors and their associates have since used this method for the mass tagging of grasshoppers in various field experiments.

Summary

First-instar nymphs of the grasshopper *Camnula pellucida* (Scudd.) fed P^{32}-treated food retained sufficient radio-activity to be readily detected with a portable Geiger counter through to the adult stage. Loss of activity through excretion was very great at first but decreased steadily to virtually nothing 14 days after treatment. Loss of activity through moulting was negligible. Survival of treated grasshoppers was as high as that of non-treated ones.

References

Fuller, R. A. 1950. Application of radio-active tracers to entomological problems. M. A. thesis. University of Saskatchewan.

Murray, D. H. 1949. Application of radio-active isotopes to insecticides. M. A. thesis. University of Saskatchewan.

Erratum

On page 158, Vol. 86, lines 20-25 should read as follows:—

11. Shape of body tadpole-like; 2 reddish-brown spots on top of head; yellow-green dorso-lateral stripe (fat bodies) on abdomen and part of thorax....*Nematinus unicolor* (Marlatt)
Shape of body not tadpole-like; markings on head as in Fig. 3; yellow-green addorsal line (fat bodies) on body; 3 yellow-green lines (fat bodies) on posterior segments ..... *Pristiphora siskiyouensis* Marlatt

First Record of the Family Deuterophlebiidae in Canada (Diptera)

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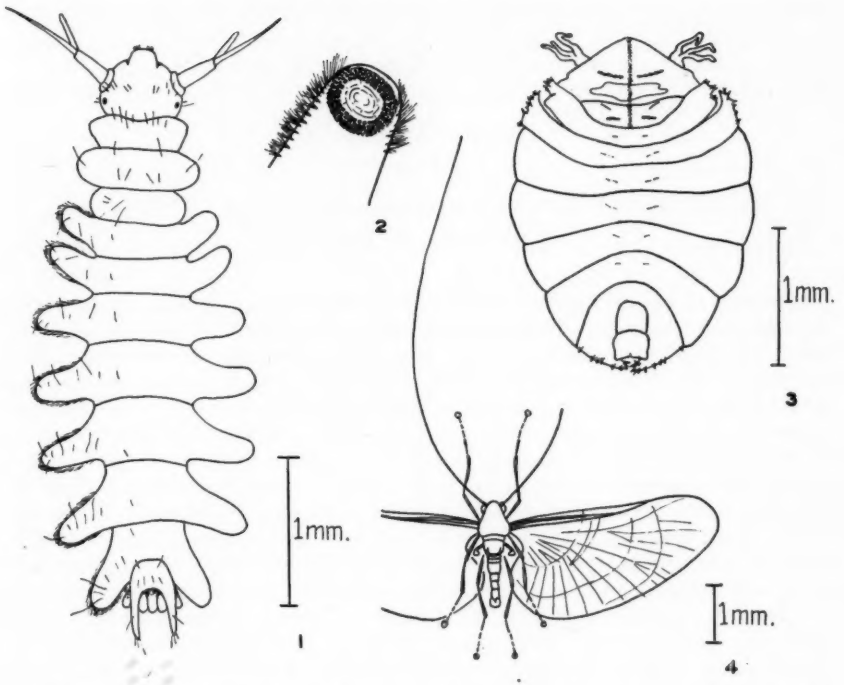
While examining the collections of immature stages of Simuliidae made at Jasper, Alta., in 1932, by Mr. J. D. Gregson, Livestock Insect Laboratory, Kamloops, B.C., I recently found a female pupa of the rare and remarkable family commonly called "mountain midges". The group consists of a single genus, *Deuterophlebia* Edwards (1922), in which four Asiatic and two North American species are recognized, although there are, also, a few records of unidentified larvae and pupae. Muttkowski (1927) first recorded the family on this continent, and Pennak (1945, 1951) and Wirth (1951) have described the two Nearctic species. Pennak (1945) has also summarized the published information on the distribution, habits, and morphology of the group.

The immature stages are found in mountain streams on the surface of rocks in broken or rippling water. (The only record of a deuterophlebiid from a large river is that of Muttkowski (1925, 1927, 1929), who took a single early-stage larva in a plankton net anchored in the Yellowstone River near its confluence with the Lamar River in Yellowstone National Park. It is possible that the specimen had been dislodged and carried into the Yellowstone from a smaller stream.). The larva (Figs. 1, 2) moves freely by means of seven pairs of abdominal prolegs furnished at their extremities with clasping organs composed of about ten concentric rings of minute claws. The pupa (Fig. 3) is broadly oval, the dorsum resembling a carapace with its margin in close contact with the rock surface, to which it adheres by means of three pairs of oval pads situated ventrally. The adults (Fig. 4) take no food and live only a few hours. They are said to emerge early in the morning and to flutter weakly above the stream surface, later falling back into the water, where their bodies may sometimes be found in large numbers floating in quiet side eddies.

The several unique characters of this family, especially those of the larva, place it in an isolated position within the suborder Nematocera. Relationship to the Blepharoceridae (net-winged midges) is suggested by a resemblance between the pupae and perhaps also by the close ecological association of the two families. Other features, however, show that the relationship is not very close. Wirth (loc. cit.) has discussed the method of attachment of the *Deuterophlebia* larva to the rock surface, pointing out that the construction of the discs on the prolegs is such that they are incapable of forming a vacuum and are therefore not to be compared with the true suction discs on the venter of the *Blepharocera* larva. This interpretation is borne out by the comparative ease with which *Deuterophlebia* larvae can be removed from the rocks. Adult mountain midges lack ocelli, mouth parts, and a true wing venation, all these being present in the Blepharoceridae.

Although most records of mountain midges are from altitudes over 5000 feet, specimens have been taken at much lower elevations. Records on this continent are from the Rocky Mountains of Colorado, Utah, and Wyoming (*D. coloradensis* Pennak) and the coastal and inland ranges of California and Oregon (*D. shasta* Wirth). In Asia, species are known from Kashmir, Japan, and the northern slopes of the Altai mountains, the latter locality at about latitude 50°.



Figs. 1-3. *Deuterophlebia coloradensis* Pennak. 1, Larva, dorsal view. 2, Tip of proleg of larva, ventral view, showing clasping organ. 3, Pupa, dorsal view. (All after Pennak, 1945).

Fig. 4. *Deuterophlebia*, adult male; part of left wing and right antenna omitted (adapted from Edwards, 1922).

The pupa here recorded, which agrees with the description of *D. coloradensis*, extends the range of the family on this continent northward over 500 miles and is the most northerly record for any species. It was taken, with several simuliid pupae, at an elevation of 6500 feet, on September 4, from a small clear stream that flows from the northeast into Cavell Creek at the foot of the Angel Glacier (I am indebted to Mr. G. H. L. Dempster, Superintendent of Jasper National Park, for interpreting Mr. Gregson's notes on this locality in the light of his special knowledge of the area). It is certainly to be hoped that entomologists of Western Canada will take the earliest opportunity to visit this or similar localities in order to procure examples of all stages of this interesting fly.

References

Edwards, F. W. 1922. *Deuterophlebia mirabilis*, gen. et sp. n., a remarkable dipterous insect from Kashmir. *Ann. Mag. Nat. Hist.*, Ser. 9, 9: 379-387.

Muttkowski, R. A. 1925. The food of trout in Yellowstone National Park. *Roosevelt Wild Life Bull.* 2: 471-497.

Muttkowski, R. A. 1927. A new and unusual insect record for North America (Diptera-Deuterophlebiidae). *Bull. Brooklyn Ent. Soc.* 22: 245-249.

Muttkowski, R. A. 1929. The ecology of trout streams in Yellowstone National Park. *Roosevelt Wild Life Ann.* 2: 151-240.

Pennak, R. W. 1945. Notes on mountain midges (Deuterophlebiidae) with a description of the immature stages of a new species from Colorado. *Am. Mus. Novitates* No. 1276.
Pennak, R. W. 1951. Description of the imago of the mountain midge *Deuterophlebia coloradensis* Pennak (Diptera, Deuterophlebiidae). *Am. Mus. Novitates* No. 1534.
Wirth, W. W. 1951. A new mountain midge from California (Diptera, Deuterophlebiidae). *Pan-Pacific Ent.* 27: 49-57.

(Received March 12, 1954)

Use of Methyl Cellulose in Laboratory Tests of Bacterial Pathogens of Insects[1]

By T. A. Angus[2]

In tests of bacteria pathogenic for defoliating insects, previous workers used water suspensions of the microorganisms and contaminated the foliage fed to the insects by dipping or spraying (1, 2). Where a quantitative result is sought such procedures have some limitations. The waxy cutin present on leaf surfaces, and this is especially true of the foliage of coniferous trees, encourages the formation of surface droplets and makes it extremely difficult to achieve uniform spreading of the bacterial suspension. In addition, the cutin interferes with the adhesion of the dried suspension to the leaf surface. To overcome these difficulties, certain adhesive agents, such as peptones, milk solids, albumin, etc., have been used, but these too are open to objection. If it is desired to reproduce as closely as possible a natural infective process, the use of protein products introduces a new variable and occasionally renders the foliage unpalatable to the insect.

In recent tests of certain *Bacillus* spp. use has been made of methyl cellulose[3] solutions. Methocel is a water-soluble synthetic gum that is chemically inert, neutral in pH, odourless, tasteless, and non-ionic. Previous toxicity tests have shown it to be harmless to plant and animal life and it has many pharmaceutical and food applications. Current results indicate that the inclusion of as much as 5 per cent Methocel in nutrient media does not inhibit growth of the *Bacillus* spp. used in tests.

Foliage coated with 1 to 5 per cent Methocel solutions was readily eaten by insects allowed to feed on it, and these solutions have been used in tests involving some 8 lepidopterous species feeding on the leaves of deciduous or coniferous trees. No marked reluctance to feed nor any change in the normal behaviour of the larvae has been noted. In such tests the amount of Methocel ingested by an insect was usually less than 0.001 gram.

It was found that 1 per cent Methocel solutions were satisfactory for most kinds of deciduous foliage, but aspen leaves, especially when very young, required the use of more viscous solutions. The bacteria being tested were suspended directly in 1 per cent solutions, or in water, in which case enough 5 per cent solution was added before use to give the desired strength. Methocel solutions may be heat sterilized and the use of such sterile solutions for suspending bacteria makes possible accurate plate counts. Most water-soluble basic dyes are compatible with Methocel, thus permitting direct microscopical examination and counting of suspended cells.

References

(1) Steinhaus, E. A. *Hilgardia* 20: 359-81. 1951.
(2) Toumanoff, C. and Vago, C. *Ann. Inst. Pasteur*, 83: 634-639. 1952.

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[3]Dow Methocel, kindly supplied by Dow Chemical of Canada, Ltd.

(Received February 15, 1954)

Some Laboratory Investigations of the Light Reactions of the Larvae of *Neodiprion americanus banksianae* Roh. and *N. lecontei* (Fitch) (Hymenoptera: Diprionidae)[1]

By G. W. GREEN[2]

Introduction

This paper deals with two sawfly species, *Neodiprion americanus banksianae* Roh. and *N. lecontei* (Fitch), important defoliators of pines in eastern North America. It is concerned chiefly with the reactions of the larvae to light and forms part of an investigation of their behaviour in response to physical factors of the environment.

Materials and Methods

Eggs of *N. banksianae* were collected early in May, 1951, from a stand of jack pine (*Pinus banksiana* Lamb.) just west of Sault Ste. Marie, Ontario. Branches containing eggs were set up in lantern jar bases, and the cut ends were immersed in water. When larvae eclosed, fresh branches were added and interwoven with the old as a readily available source of fresh food. The same procedure was followed in subsequent food changes.

Late-stage larvae of *N. lecontei* were collected at South River, Strong Township, Ontario, in September, 1950. The larvae were reared on red pine (*Pinus resinosa* Ait.) and the ensuing cocoons were placed in cold storage for the winter. The adults from these cocoons formed the parent stock for larval populations of *N. lecontei* used in this study. Foliage changes as the larvae developed were made as described above.

To augment the laboratory stock, additional larvae were collected as required from field populations at various stages of development. Rearing room conditions for all stocks were maintained between 20 and 21°C. and 60 and 79% R. H. throughout the developmental period.

Laboratory investigations were concerned chiefly with the reactions of both species to point sources of light and with the effect of temperature upon responses to diffuse light.

The type of board employed by Wellington (2) in work with the spruce budworm was not suitable for use with sawfly larvae, for, indoors, they usually move quite sluggishly on flat surfaces, and can be tested only on a relatively small board. This limited the size of the lamp used since it has been suggested (2) that a 40 or 60-watt lamp close to an insect may appear as a patch of diffuse light, instead of a point source, because of the very great difference in the size of the insect's head and the lamp. Consequently, a small, six-watt, panel lamp was mounted in the centre of a 28-inch square of black cardboard, which formed the reaction platform. The platform was scribed with concentric reference rings spaced one inch apart and centred on the lamp. The platform was covered with a sheet of clear cellulose acetate since small larvae of both species move more easily over this type of surface than over cardboard. A 12-inch wall of black cardboard surrounded the platform and excluded light from another small lamp, which was used to illuminate the record sheets.

Tests were made with larvae taken directly from feeding colonies and with larvae that were starved for six hours in empty rearing jars under room conditions. Larvae were placed on the platform at intervals around the 4-inch reference ring. It was possible to test 10 larvae of the younger instars at a time,

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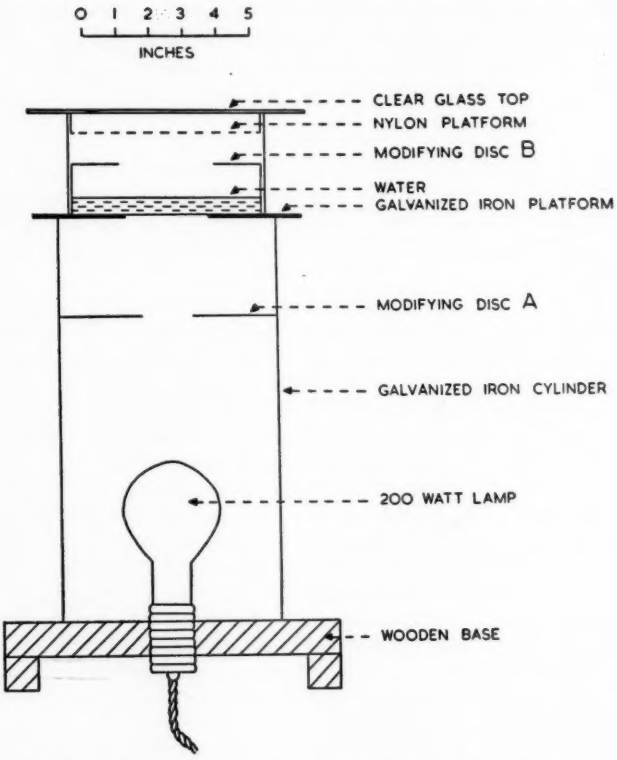

Fig. 1. The dark-light alternative chamber used to determine the temperatures at which larvae of *N. lecontei* became negative to diffuse light.

but as they grew larger and more active, smaller numbers had to be used in each test. Diagrams of the paths followed by larvae during their response to point-source stimulation were made during each observation. After 15 minutes of exposure, a larva was considered to be indifferent to point-source stimulation if it had not moved the four inches to the light source, had not performed compass reactions, or had not exhibited a photonegative response.

Two types of dark-light alternative chambers were used in experiments to determine the effects of temperature on the orientation of larvae to diffuse light. The first type, used in experiments with *N. banksianae*, has been described by Wellington (2) and, more recently, by Sullivan and Wellington (1). Using this type of chamber, the reversal temperature recorded for each larva was the temperature at which it entered the dark and remained therein. If two or more larvae entered the dark, where they were lost from view, at temperatures within the reversal range, and then one of them returned to the light again, it was impossible to decide which of the recorded reversal temperatures was invalid. Hence, in this apparatus, a large number of larvae could not be tested at one time. However, if only five larvae were used in each test, this difficulty was usually eliminated. On this basis, five larvae per experiment were adopted as standard.

Studies with *N. banksianae* showed that, apart from the problem noted above, this type of alternative chamber was not altogether satisfactory for work with sawfly larvae. Small larvae, especially, became disorientated when they moved far into either the light or the dark portion of the chamber, and they either experienced some difficulty in locating the dark-light boundary again or else took too long to reach it in response to an increase in temperature. Therefore, when larvae of *N. lecontei* were tested, a new type of alternative chamber was employed.

The new chamber is shown in Fig. 1. It was designed to increase the length of the dark-light boundary and to decrease the distance that a larva need travel from any point in the chamber to reach the boundary. Moreover, the amount of observer error associated with the former chamber was greatly reduced because larvae were always observable at any position in the chamber.

A 200-watt, Mazda lamp, mounted on a wooden base, was enclosed in a cylinder of galvanized iron, 12 inches high and 6½ inches in diameter. The lamp provided both heat and light for the apparatus. A galvanized iron disc (A) was situated part way up the cylinder. This disc had a 1½-inch hole in its centre, and fitted snugly to the inside of the cylinder, in which it could be moved up or down to modify the lighted portion of the reaction chamber above it. The galvanized iron platform upon which the reaction chamber rested was slightly larger than the cylinder and had a 2-inch hole in its centre. The reaction chamber was a 2000 cc. culture dish, fitted inside with a galvanized iron sleeve to which was soldered the final modifying disc (B). This disc had a 2-inch hole in its centre and was situated 1½ inches above the bottom of the reaction chamber. A nylon mesh platform upon which the insects moved about the chamber was held in place 1 inch above modifying disc (B) by an adjustable aluminum ring as described by Wellington (2). The chamber was covered with a piece of clear glass. With the modifying discs arranged as above, a 3-inch circle of light, surrounded by deep shadow, could be thrown on the nylon platform. All metal parts of the apparatus were painted a dull black to keep light reflection at a minimum.

Before the start of an experiment, modifying disc (A) was moved up or down the cylinder until the patch of light thrown on the nylon platform was best suited to the size of the larvae being tested. The lamp was then left on to allow the apparatus, with the exception of the reaction chamber, to warm. While heating proceeded, the sleeve with modifying disc (B) was removed from the reaction chamber and the latter filled with cold tap-water to the desired level. The water level depended upon the rate of temperature rise desired. In general, one-half inch of water was used. This gave a temperature rise of nearly one degree Centigrade per minute. As described (2) for the older type of alternative chamber, water was used so that the air in the reaction chamber was always saturated. This procedure eliminated increasing rates of evaporation as temperatures rose so that any changes observed in the reactions could be attributed to temperature *per se*.

The sleeve was then replaced in the reaction chamber, and the nylon platform set into position and smoothed. With the exception of instars V and VI, 20 larvae were placed in the chamber at the start of each experiment. Fewer larvae of later instars were tested in each experiment because of their larger size.

To prevent condensation in droplet form, a coating of thick soap solutioon was applied to the inside of the glass plate that formed the lid of the apparatus.

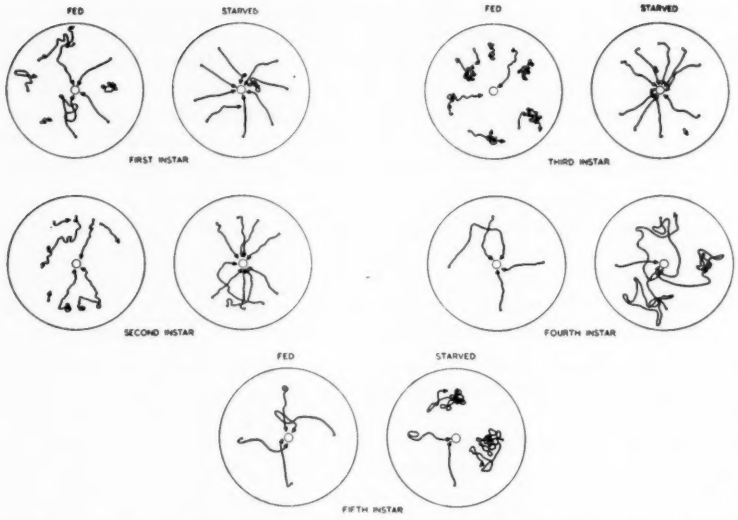

Fig. 2. Representative paths taken by fed and by starved larvae of *N. banksianae* in response to a six-watt lamp.

The glass plate was then wiped with a dry paper towel and a very thin layer of soap was left on the inside of the plate. After treatment in this manner water droplets never formed on the glass and visibility into the apparatus was considerably improved.

As with the former type of alternative chamber, temperature measurements were made with a No. 24 copper-constantan thermocouple centrally located in the chamber with the junction resting lightly upon the nylon reaction platform. The thermocouple was used in conjunction with a "Rubicon" portable potentiometer connected to a sensitive external galvanometer. With each one-degree change in temperature, a record was made of the number of larvae in the lighted portion of the chamber. Most of the experiments were started at room temperature, and the temperature within the chamber was raised slowly until all of the larvae had entered the dark. Thirty minutes, corresponding to a temperature rise of approximately 25°C. was usually sufficient to complete one experiment.

Results

Reactions to a point source

The reactions of larvae of *N. banksianae* to a six-watt lamp are shown in Table I. Typical paths followed by larvae in response to the lamp are shown in Fig. 2.

When removed from a feeding colony and exposed to a point source, *N. banksianae* larvae in the first three instars were, for the most part, indifferent to the light. After six hours' starvation, however, they became strongly photopositive. On the other hand, larvae in the last two feeding instars were strongly positive to light from a point source directly upon removal from feeding colonies. Starvation made no significant difference (2x2 contingency – $P > 0.10$) in the

TABLE I
Reactions of larvae of *N. banksianae* to a six-watt lamp at room temperature

Instar	Condition	Reaction		No. tested
		Positive (%)	Indifferent (%)	
I	Fed	38.9	61.1	54
	Starved	85.1	14.9	47
II	Fed	12.0	88.0	25
	Starved	91.2	8.8	57
III	Fed	33.4	66.6	45
	Starved	87.5	12.5	40
IV	Fed	90.0	10.0	50
	Starved	77.7	22.3	36
V	Fed	81.3	18.7	32
	Starved	84.6	15.4	52

reaction of these older larvae, although the paths they made to the light when starved were more convoluted than when fully fed.

Table II and Fig. 3 present similar data for larvae of *N. lecontei*. Like *N. banksianae*, first-instar larvae of *N. lecontei* were, for the most part, indifferent to light from a point source directly upon removal from feeding colonies, but when starved for six hours, they became strongly photopositive. Unlike *N. banksianae*, however, the indifference noted in young, well-fed larvae did not extend to the second and third instars. Instead, instars II and III of *N. lecontei*

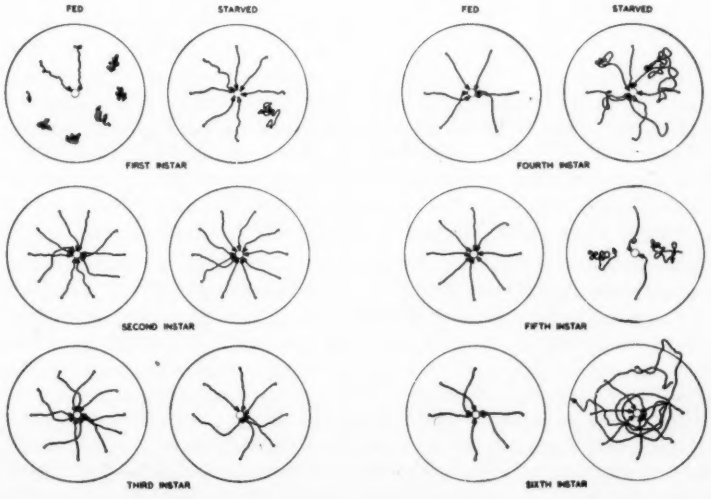

Fig. 3. Representative paths taken by fed and by starved larvae of *N. lecontei* in response to a six-watt lamp.

were strongly photopositive to light from a point source directly upon removal from the feeding clusters, and starvation for six hours produced no change in their response. The last three feeding instars of this species were also strongly positive to point-source light when well fed, but starvation for six hours resulted in more larvae reacting indifferently. Contingency tests show that the differences observed between fed and starved larvae of the fifth and sixth instars are significant and on the verge of significance in instar IV. In addition, although many of these later-stage larvae remained positive when starved, their paths to the light were much more convoluted than when well-fed, and many of them performed light compass reactions (Fig. 3).

In neither of the species, at room temperature, was any truly photonegative behaviour observed.

TABLE II
Reactions of larvae of *N. lecontei* to a six-watt lamp at room temperature

Instar	Condition	Reaction		No. tested
		Positive (%)	Indifferent (%)	
I	Fed	15.7	84.3	42
	Starved	97.4	2.6	39
II	Fed	97.5	2.5	40
	Starved	97.4	2.6	39
III	Fed	97.4	2.6	39
	Starved	98.1	1.9	51
IV	Fed	98.0	2.0	50
	Starved	85.7	14.3	35
V	Fed	97.9	2.1	49
	Starved	75.0	25.0	32
VI	Fed	96.0	4.0	50
	Starved	80.0	20.0	33

Fifth-instar larvae of *N. lecontei* were starved for periods of 24 and 72 hours to see if this had any effect upon their reaction to point-source light. Starvation for 24 hours increased the degree of indifference and made many more larvae exhibit modified compass reactions. Starvation for 72 hours resulted in a reversal to the strongly photopositive response observed in fully-fed larvae.

Virus disease affected the reactions of *N. lecontei* to point-source light. Twenty fourth-instar larvae suspected of being virus-infected were removed from the rearing stock and kept on red pine foliage in two groups of 10 each. These larvae were tested on the light board once a day. The paths followed by larvae of one of these groups on successive days as the disease developed are shown in Fig. 4. At first, all of the larvae were strongly photopositive. At this stage, they all looked healthy, and a good deal of frass was present in the rearing jar. When tested next day (Aug. 16) nine of the larvae reacted photopositively and one was indifferent. Those that reacted positively did so less directly than on the previous day. At this stage, there was a reduction in the amount of frass in the rearing jar, but all of the larvae still appeared to be healthy. On Aug. 17, one of the larvae had moulted to the fifth instar. Many of the larvae were quite

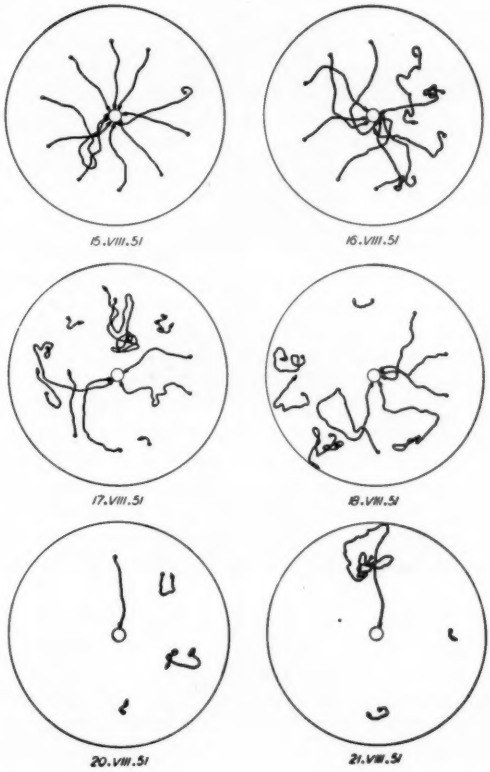

Fig. 4. Representative paths taken by virus-infected late-stage larvae of *N. lecontei* in response to a six-watt lamp on successive days during the development of the disease.

translucent, and little food could be seen in their guts. More were indifferent than in previous tests. On Aug. 18, the reaction to light was much the same as on the previous day, but, of three more larvae that had moulted to the fifth instar, one was almost dead—not moving from where it was deposited on the board. By Aug. 20, six of the larvae were dead and turning black. The remaining four were in the fourth instar, and when tested, only one reacted photopositively. This larva did not appear to be diseased, and had plenty of food in its gut. By the next day, another larva was dead, and two were indifferent to the stimulus and almost dead. The larva that had been positive the previous day was positive again, but reached the light by a more convoluted path than previously. By Aug. 22, all the larvae in the group were dead, except for this last positive one. It continued to develop and finally spun a cocoon. The other group tested reacted in virtually the same manner as described above.

Reactions to diffuse light

Larvae of both species were acclimated to 20-21°C. and 60-79% R. H. by rearing them under controlled room conditions. At room temperatures, larvae in all feeding instars, of both species, were quite positive to diffuse light if this were presented to them in gradient form. If they were given a choice between a lighted and a darkened portion of an alternative chamber, however, their reactions at room temperatures were much less precise, and, at first glance, they appeared to be indifferent to diffuse light. However, when their movements around the chamber were observed at length, it soon became evident that they spent much more time in the light than in the dark.

At room temperatures, larvae crossed and recrossed the dark-light boundary with no indication that they perceived its presence, but when the temperature was slowly raised to higher levels, although they still journeyed across the boundary, they gave decisive evidence that they perceived it. At temperatures approaching 30°C., two main boundary reactions were observed. The larvae often approached the boundary, stopped briefly upon reaching it, and searched about with active head movements Then they either entered the dark or turned back into the light again. Both movements were sharp and decisive. In many cases, however, the reaction took a different form. Larvae upon reaching the boundary, aligned their bodies with it and moved along it. If larvae happened to be in the dark portion of the chamber at these temperatures, their return to the light was usually rapid and direct, with no pause at the boundary. It was quite evident that the insects perceived the boundary at higher than room temperatures, and their reactions were, on the whole, strongly photopositive at these temperature levels.

In both species, agitated movements began when the reversal range of temperature was approached. Movement into the darkened portion of the chamber was usually rapid and distinct for any one individual, although the appearance of the group at these levels gave the impression of a general milling movement near the boundary. Larvae sometimes returned to the dark-light boundary after entering the dark at temperatures within the reversal range, but they rarely ventured over the boundary and into the lighted portion of the chamber again. If they did so, it was for very short trips only, after which they re-entered the dark and moved far back into it, their entire response definitely reversed from the strongly photopositive reaction at slightly lower temperatures.

Although the general reactions of both species were markedly similar, the two have to be considered separately since two entirely different types of alternative chambers were used, and the manner of recording the results in each makes the same statistical treatment impossible.

Reversal temperatures of larvae of *N. banksianae* are shown in Fig. 5, which illustrates the frequency distributions obtained when 100 fully-fed larvae of each instar were tested in the alternative chamber. A broad reversal range exists for each of the instars. These ranges vary between six and nine degrees Centigrade. Since the light reactions of these insects are, on the whole, rather weak, such ranges are to be expected.

The mean reversal temperatures for the feeding instars of *N. banksianae* are presented in Table III. Successive pairs of these means were subjected to *t* tests. These tests indicated no significant difference between the reversal temperatures of instars I and II ($P=0.50$), but each subsequent instar differed significantly ($P<0.05$) from the preceding instar. It is interesting to note that the mean reversal temperatures of instars I and II are the highest in the series measured.

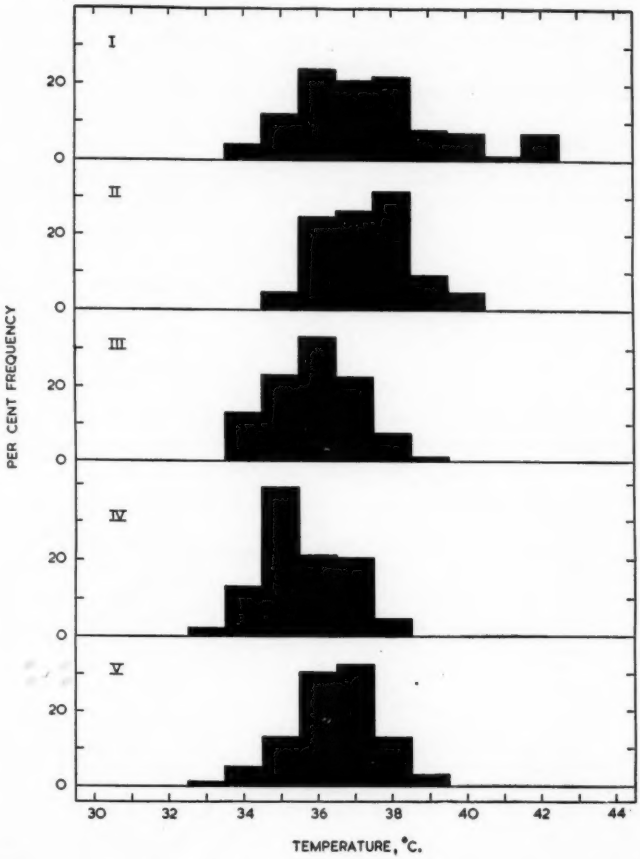

Fig. 5. Inter-instar comparison of the ranges of temperature at which larvae of *N. banksianae* became photonegative to diffuse light in saturated air after acclimation to 20.5°C.

TABLE III

Comparison of the mean temperatures at which fed larvae of *N. banksianae* became photonegative to diffuse light in saturated air after acclimation to approximately 20.5°C.

Instar	I	II	III	IV	V
n	100	100	100	100	100
$\bar{x}$	37.2	37.3	35.9	35.5	36.4
$s_{\bar{x}}$	0.194	0.121	0.126	0.108	0.141

n = sample size; $\bar{x}$ = mean reversal temperature, °C; $s_{\bar{x}}$ = standard error of the mean

With the exception of instar I, larvae of *N. banksianae* were tested in the alternative chamber after being starved for approximately six hours. The mean

temperatures at which starved larvae became photonegative are presented in Table IV. No significant difference exists (P=0.90) in the mean reversal temperatures of fed and starved second-instar larvae. All other instars have significantly *lower* reversal temperatures when starved.

TABLE IV

Comparison of the mean temperatures at which starved larvae of *N. banksianae* became photonegative to diffuse light after acclimation to approximately 20.5°C.

Instar	I	II	III	IV	V
n	–	50	50	50	50
$\bar{x}$	–	37.4	33.9	34.9	35.3
$s_{\bar{x}}$	–	0.18	0.14	0.13	0.30

n = sample size; $\bar{x}$ = mean reversal temperature, °C.; $s_{\bar{x}}$ = standard error of the mean

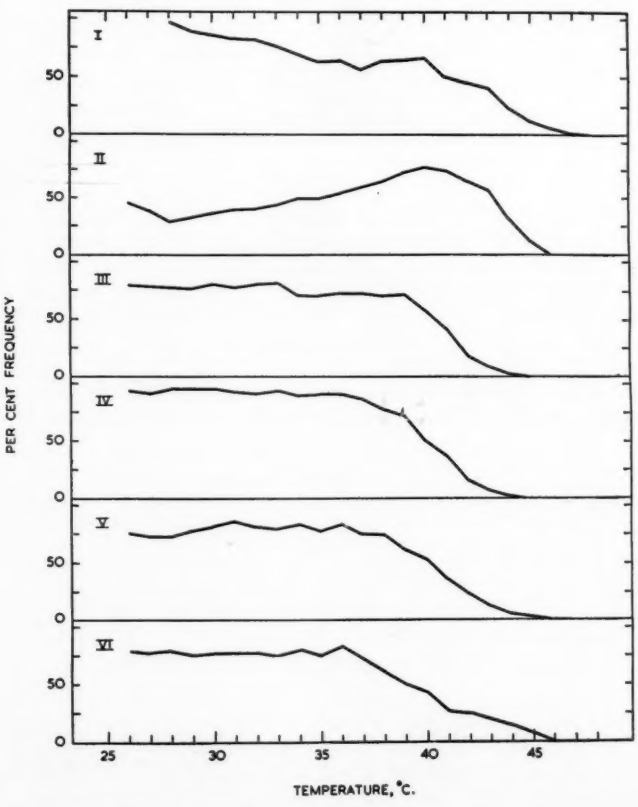

Fig. 6. Percentage frequency of *N. lecontei* larvae of instars I to VI photopositive to diffuse light at different temperatures.

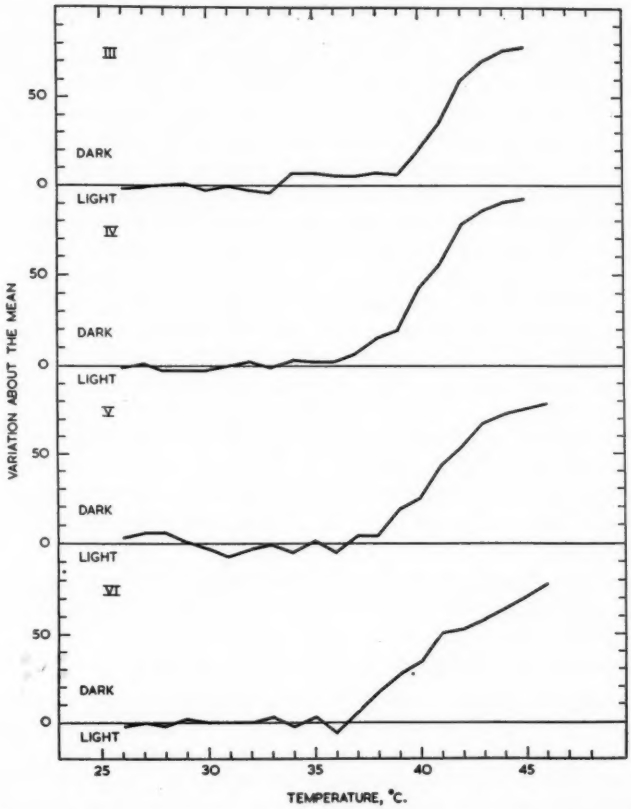

Fig. 7. Variations with temperature in the number of *N. lecontei* larvae of instars III to VI inclusive, in the lighted portion of the alternative chamber, about a mean calculated for larvae in the light at temperatures between 26 and 35°C.

Since larvae of *N. lecontei* were tested in the modified type of alternative chamber earlier described, the analysis of the resulting data and the manner of their presentation require some discussion.

The percentages of larvae in the lighted portion of the chamber are plotted against temperature in Fig. 6. With the exception of instars I and II, relatively constant numbers of larvae of each instar were found in the lighted portion of the chamber at temperatures below 35°C. This number never reached 100% of the population tested, because larvae were continually moving back and forth across the dark-light boundary at temperatures below the reversal range. Although their journeys into the dark were usually of short duration, the constant interchange of larvae across the border resulted in some of them being in the darkened portion of the chamber at all temperatures. About 10-20% of the population could be expected in the dark at all times, so that selection of a 50% reversal point from the curves shown in Fig. 6 would not be a true value for the population. Some arrangement of the data had to be made to correct this error and it was made as follows.

The data for instars I and II are not considered for the time being so that the argument is confined to instars III to VI inclusive. Fig. 6 reveals a fairly constant number of insects of each instar in the light at temperatures below 35°C. The mean numbers of insects in the light between 26 and 35°C. were calculated for each of the instars under consideration. The variations in numbers of insects about these means were then calculated and are shown graphically in Fig. 7. It is evident that the beginning of the reversal range in each case must be considered as the point at which the number of larvae in the lighted portion of the chamber never becomes greater than the mean number in the light at temperatures between 26 and 35°C. Examination of the curves in Fig. 7 for instars III and IV reveals for each, a fairly broad but shallow plateau just above the base line before strong and steady movements into the dark occur. It may be argued that these plateaus are not real, and that, had the mean and twice its standard error been used as the base line in each case, these plateaus may have disappeared and consequently the start of the reversal ranges shifted to higher positions in the temperature scale. The standard errors of the means for these two instars were calculated and the corrections applied. In both cases, the plateaus remained above the newly established base lines. Hence, the reversal ranges begin where the curves in Fig. 7 start to rise consistently above their base lines. On this basis, the lower limits of the reversal ranges fall at 34°C. for instars III and IV, and at 37°C. for instars V and VI.

It must be understood that the temperatures quoted as the beginnings of the reversal ranges have not been pointed up for the biological significance that they may have. They have been stated only to form a logical starting point from which calculations of the 50% reversal points for each instar may be made.

The results for instars I and II are not amenable to this approach. These smaller larvae consistently moved away from the brush used to place them in the alternative chamber: an avoiding reaction which masked their actual photic response for some time. This reaction resulted in many larvae moving into the darkened portion of the chamber at temperature ranges within which they normally would have been photopositive. As the temperature within the chamber was raised, however, they began to return to the lighted portion of the chamber again and remained therein, until a steady progression into the dark began, in the neighbourhood of 40°C. The curves for instars I and II in Fig. 6 indicate that 40°C. is the logical lower limit of the reversal ranges of these two instars, and that the reactions exhibited before this temperature was reached were not truly photic responses. The same type of reaction may be observed in these younger instars when they are disturbed in the feeding cluster. The entire colony invariably migrates inward from the feeding site to the branch or the main stem and may stay there up to an hour after the disturbance ceases.

With the lower limits of the reversal ranges established, cumulative percentage frequencies of larvae moving into the darkened portion of the chamber at temperatures within the reversal ranges were calculated and plotted (Fig. 8). Mean reversal temperatures were calculated from these data and are presented in Table V where they are compared with the 50% reversal points indicated in Fig. 8. The 50% reversal points and the mean reversal temperatures are seen to correspond quite closely. Therefore, in future work using this method of establishing the reversal ranges, calculations beyond the level shown in Fig. 8 would appear unnecessary.

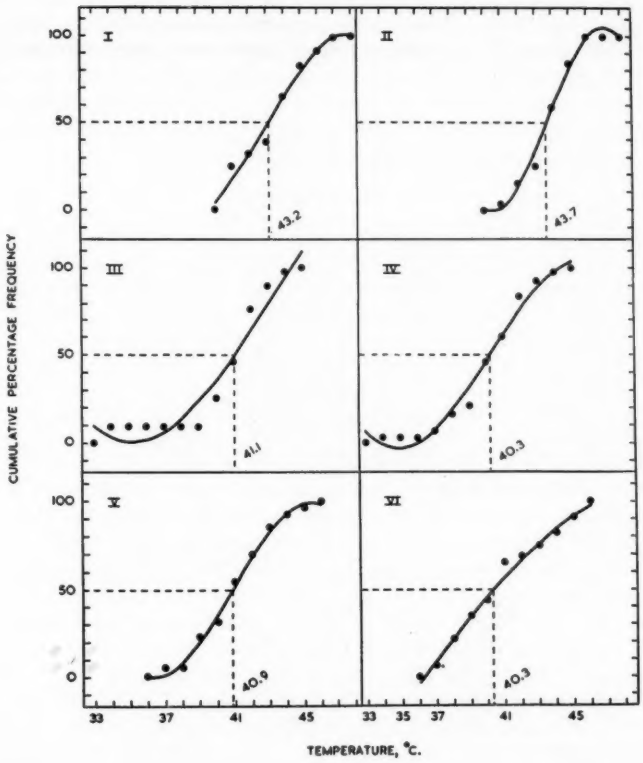

Fig. 8. Cumulative percentage frequency curves for larvae of *N. lecontei* moving into the dark at temperatures within their respective reversal ranges: polynomials of the second degree fitted. The broken lines represent 50 per cent reversal points.

TABLE V

Comparison of the mean temperatures at which fed larvae of *N. lecontei* became photonegative to diffuse light in saturated air after acclimation to approximately 20.5°C.

Instar	I	II	III	IV	V	VI
n	100	100	100	100	85	40
$\bar{x}$	43.7	44.1	41.1	40.7	41.4	41.1
$s_{\bar{x}}$	0.21	0.13	0.25	0.23	0.46	0.29
50% reversal levels (Fig. 8)	43.2	43.7	41.1	40.3	40.9	40.3

n = sample size; $\bar{x}$ = mean reversal temperature, °C.; $s_{\bar{x}}$ = standard error of the mean

Successive pairs of the mean reversal temperatures presented in Table V were subjected to *t* tests. No significant difference ($P>0.05$) exists between instars I and II. Instar III reverses at a significantly lower temperature ($P<0.01$) than instar II, but each successive comparison of means showed no difference except between instars IV and V, which were just within the significant range (P circa 0.03). Again, as with *N. banksianae*, the mean reversal temperature of instars I and II are the highest in the series measured.

Discussion

Wellington (2) has shown that starvation of late-instar spruce budworm larvae produces a light compassing reaction that changes to truly photonegative behaviour as the starvation period is prolonged. In apparent agreement with this, six-hour starvation of late-instar sawfly larvae resulted in a greater degree of indifference than was observed in fully fed larvae of comparable development. Because of this increase in indifference, significantly fewer larvae of *N. lecontei* in instars V and VI reached a light source during test periods, and, in addition, many of those that were recorded as reacting in a positive fashion did so by a light compassing reaction. Although there was no significant difference in the numbers of late-instar larvae of *N. banksianae* reaching the light after short-term starvation, the paths followed by starved insects were much more convoluted than those of fully fed individuals. These results suggest that the difference in reaction between sawfly and spruce budworm larvae is a matter only of degree, and that prolonged starvation of late-stage sawfly larvae might eventually result in a photonegative response. This, however, is not the case with larvae of *N. lecontei*, at least. Prolonged starvation of this species results in a final reversion to a strongly positive reaction to point-source light.

In contrast to the indifference and compassing resulting from short-term starvation of late-instar sawfly larvae, the early instars of *N. banksianae* and the first instar of *N. lecontei* reverted from a high degree of indifference to a strongly photopositive response after starvation. Therefore, a change must take place in the visual response complex of sawfly larvae as they develop through successive instars. At first, short-term starvation results in a strongly photopositive response, and in later instars it produces either a compassing reaction or an increase in indifference.

The effects of starvation on the photic responses of sawfly larvae may have an important bearing on field behaviour. Larvae of *N. banksianae* and *N. lecontei* have been kept under observation in the field immediately following eclosion. After the head capsules darken and harden, newly-emerged larvae begin to move about on the foliage. This movement continues until several larvae come in contact with each other, whereupon they usually form a compact feeding group. Although the wanderings of these larvae before the formation of feeding groups appears to be at random, the overall direction of their travels is definitely photopositive as far as movement from one needle to the next is concerned. Consequently, strongly photopositive behaviour at this stage is advantageous to the rapid formation of feeding colonies by larvae from one egg cluster. Reacting photopositively, larvae automatically head towards the end of the branch, thereby reducing, by at least one-half, the amount of foliage they might wander over before forming a feeding group. On the other hand, by the time larvae of a colony have reached the fourth instar, the branch upon which they have been feeding is often stripped of edible foliage. Their greater degree of indifference to light at this stage allows starving larvae to range more widely over the tree in search of a new feeding site than if they had remained rigidly photopositive.

The increase in the degree of indifference to point-source light exhibited by virus-infected larvae of *N. lecontei* seems to be a result of the emptying of the gut of solid food material as the disease develops, since the light reactions associated with the development of the disease are comparable to the effects of short-term starvation.

First- and second-instar larvae of both species exhibit reversal temperatures that are considerably higher than those of subsequent stadia. This is not always the case (cf. (1) and (2)) where the temperatures at which reversal occurs increase with larval development in agreement with general increases in ambient air temperatures through the developmental period. The possible significance of the higher reversal temperatures in the early instars of sawfly larvae is not yet fully understood, but it may be associated with the feeding behaviour of the younger larvae. In addition to the differences noted above, the overall reversal temperatures of larvae of *N. banksianae* are lower than those of *N. lecontei*. This might be expected, since larvae of *N. banksianae*, active in the early spring, are exposed to lower ambient air temperatures than are larvae of *N. lecontei*, which are active later in the warmer parts of the year, and frequently feed on smaller trees that are exposed to the layer of warmer air near the ground.

Starvation appears to affect temperature stimulation of the visual response complex in larvae of *N. banksianae*. Starved larvae of this species exhibit reversal temperatures that range from 0.6 to 2.0°C. *lower* than fully fed larvae. The reason for this difference is still obscure.

Summary

1. Fed *N. banksianae* larvae of the first three instars are, on the whole, indifferent to discrete light at room temperatures. When starved for six hours they become strongly photopositive. Fully fed fourth- and fifth-instar larvae of this species are positive to discrete light at room temperatures. The effect of short-term starvation on these later stages is evident in the more circuitous paths they follow in their positive response to a point source.

2. First-instar larvae of *N. lecontei* are indifferent to discrete light when fully fed, and once again, starvation for six hours results in a strongly photopositive response. All other instars are positive to discrete light at room temperatures when fully fed, but some differences appear after starvation. Short-term starvation does not change the sign of the reaction of second- and third-instar larvae but it does produce both a greater degree of indifference and some light compassing reactions in the last three instars.

3. Larvae of *N. lecontei* infected with a polyhedral virus disease become increasingly less positive to point-source light as the disease becomes established. This increasing indifference is comparable to the effects of short-term starvation of healthy larvae.

4. Prolonged starvation of late-stage larvae of *N. lecontei* up to 24 hours results in increased indifference and some compassing in response to point-source light. Further starvation to 72 hours results in a reversion to strongly photopositive behaviour.

5. Fed *N. banksianae* larvae of the first- and second-instars become photonegative to diffuse light between 37.2 and 37.3°C. Third-, fourth-, and fifth-instar larvae of this species become photonegative between 35.5 and 36.4°C. When starved for six hours, *N. banksianae* larvae of the second and subsequent instars become photonegative to diffuse light at *lower* temperatures than when

fully fed. This decrease in reversal temperatures is significantly different in instars III to V inclusive.

6. Fully fed *N. lecontei* larvae of the first- and second-instars become photonegative to diffuse light between 43.7 and 44.1°C. All other instars become photonegative between 40.7 and 41.4°C.

Acknowledgments

The author wishes to thank M. L. Prebble, R. M. Belyea, and members of the staff of the Forest Insect Laboratory, Sault Ste. Marie, Ontario, for their helpful criticism and advice. This work was done under the supervision of W. G. Wellington, whose help and guidance are gratefully acknowledged. Thanks are due Miss P. M. G. West for technical assistance in the laboratory.

References

(1) Sullivan, C. R. and W. G. Wellington. 1953. The light reactions of larvae of the tent caterpillars, *Malacosoma disstria* Hbn., *M. americanum* (Fab), and *M. pluviale* (Dyar) (Lepidoptera: Lasiocampidae). *Can. Entomol.* 85: 297-310.

(2) Wellington, W. G. 1948. The light reactions of the spruce budworm, *Choristoneura fumiferana* Clemens (Lepidoptera: Tortricidae). *Can. Entomol.* 80: 56-82A.

(Received March 3, 1954)

Spalangia rugosicollis Ashm. (Hymenoptera: Chalcidoidae), a New Parasite of the Onion Maggot, *Hylemya antiqua* (Mg.) (Diptera: Anthomyiidae)

By J. P. Perron

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Early in September, 1953, an examination of 370 first-generation pupae of the onion maggot, *Hylemya antiqua* (Mg.), collected from large field rearing cages at St. Jean, Que., revealed that 41, or 11.1 per cent, were parasitized. The parasite was identified as *Spalangia rugosicollis* Ashm. by Dr. O. Peck, Systematic Entomology Unit, Entomology Division, Ottawa. Dr. Peck (in litt.) stated that this is the first record of this parasite attacking the onion maggot and that the Nearctic species in this genus have been greatly confused.

When the observations were made, the parasites were beginning to emerge and a few pupae of the onion maggot still contained the immature stages of the parasite. Only one parasite developed from each pupal host, and only first-generation pupae were parasitized.

(Received March 23, 1954)

Studies on Dispersal of Grasshoppers (Acrididae) Tagged with Phosphorus-32[1]

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The main objective of the present work was to investigate the ability of grasshopper nymphs to escape an environment devoid of food plants and to reach a suitable food supply, either as a result of random dispersal or by marching. The immediate interest was in movement over recently tilled surfaces. It was also desired to investigate the external factors that might influence such movement.

Munro and Telford (1942) traced the movements of third- to fifth-instar grasshoppers for distances up to 100 yards across barren, cultivated fields in less than 2½ hours. Post and Anderson (1950) observed the movement of second-instar nymphs of *Melanoplus* spp. up to 100 feet in 24 hours. Furthermore, the ability of nymphs of *Camnula pellucida* (Scudd.) and *Melanoplus mexicanus mexicanus* (Sauss.) to move considerable distances while destroying seedling crops is a common observation made by most field men. Such results suggested that many nymphal grasshoppers could easily escape from a field suddenly made barren by tillage.

Nevertheless, in field experiments with grasshopper infestations in cultivated fields, the junior author had often failed to detect significant movement of grasshopper nymphs off barren plots as small as 2 to 8 acres in size. Attention was especially drawn to this where newly hatched nymphs emerged in bare, tilled fields. Their apparent failure to move "purposefully" resulted in starvation and death.

The use of a radio-active tag promised to be of great aid in tracing the movements of immature grasshoppers in the field. The work of Fuller *et al.* (in press) indicated the potential usefulness of phosphorus-32 for this purpose. It was adopted as more suitable for moulting nymphs than a superficial tag, such as the red lacquer used by Munro and Saugstad (1938) on migrating adults, or the fluorescent lacquer used on nymphs by Post and Anderson (1950).

Methods

Essentially the same procedures were used for four separate field releases. Each release was made on a bare, weed-free, summer-fallowed field. The first two fields were situated near Davidson, the third and fourth east of Saskatoon, Sask. The grasshoppers were released near the centres of the fields, which varied in size from 2.5 to 80 acres. The nearest green vegetation or growing crop was 45 to 225 yards from the release points. Indigenous grasshopper populations were extremely low in most instances.

Tagging

Wheat seedlings, growing to a height of about 6 inches in a 2- by 2-foot flat, were used as the food carrier for the P^{32}. The plants were sprayed with about 50 cc. of a solution containing 0.5 mc. of the radio-active element. This operation was done in the open field with a manually operated atomizer. After

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the solution had dried on the foliage, a wire screen cage was placed over the flat, which had been built to fit this purpose, and the grasshoppers were placed in the cage.

Some mortality occurred, especially before the first two releases. Overcrowding and packing between the walls of the cage and the sides of the flat killed many first- and second-instar grasshoppers. This led to the use of two cages instead of one in the third and fourth trials.

To obtain maximum utilization of the P^{32}, the aim was to place enough grasshoppers in the cage to consume all the treated food in a short time. Accordingly, several thousand grasshopper nymphs or adults (Table II), stored overnight without food during the immediately preceding period, were caged. After several hours, when the food was consumed, a representative sample of about 20 grasshoppers was removed, and their individual radio-activity counts were determined with a portable survey meter (Model 2610A, Nuclear Instrument Company, Chicago, Ill). The cage was then removed, releasing the mass of radio-active grasshoppers.

Tracing Dispersal Patterns

Grasshoppers were collected with a sweep-net or by hand about the periphery of the dispersal area at various intervals after each release. The perimeter of dispersal of the grasshoppers was established by sampling while approaching the release point from four or more directions. Collections in any given direction were halted as soon as radio-active grasshoppers were encountered. Hence, disturbance caused by activities of the observers was kept at a minimum within the main dispersal area. Distances and rates of movement quoted are based on the distance of the perimeter from the release point. Such figures do not take into consideration the movement of all grasshoppers, many of which were probably much nearer the release point than those at the perimeter.

In the sampling by the sweep-net method, one or more radio-active grasshoppers were easily detected by monitoring the net with the Geiger tube, even when untreated, indigenous grasshoppers were taken. Radio-active individuals were quickly separated from mass collections when desired. All radio-active grasshoppers caught were returned uninjured to the point of collection after monitoring.

Seven days after the fourth release, all the grasshoppers within 300 yards of the release point were collected. All individuals were monitored and their positions plotted in relation to the release point. Thus, quantitative information on the numbers surviving under starvation conditions was incidentally obtained.

Recording Weather Data

A soil thermograph and an air hygrothermograph were set up at ground level at the release site, and continuous temperature and relative humidity records were obtained. The bulb of the soil thermograph, held horizontally, was pushed into the soil so that the upper surface of the bulb was at ground level and then covered with a thin film of dusty soil. Soil temperatures were indicative of the mean temperature in about the top inch of soil, but also approximated the soil surface temperature. The temperature in the immediate environment of most grasshoppers, and in the bodies of individuals, may frequently have exceeded recorded soil surface temperatures. Observations on wind velocity, duration, and direction were made regularly during and irregularly between collection periods.

In addition to total time, dispersal activity was related to periods during which the temperature was 70°F. or higher. This was assumed to be the

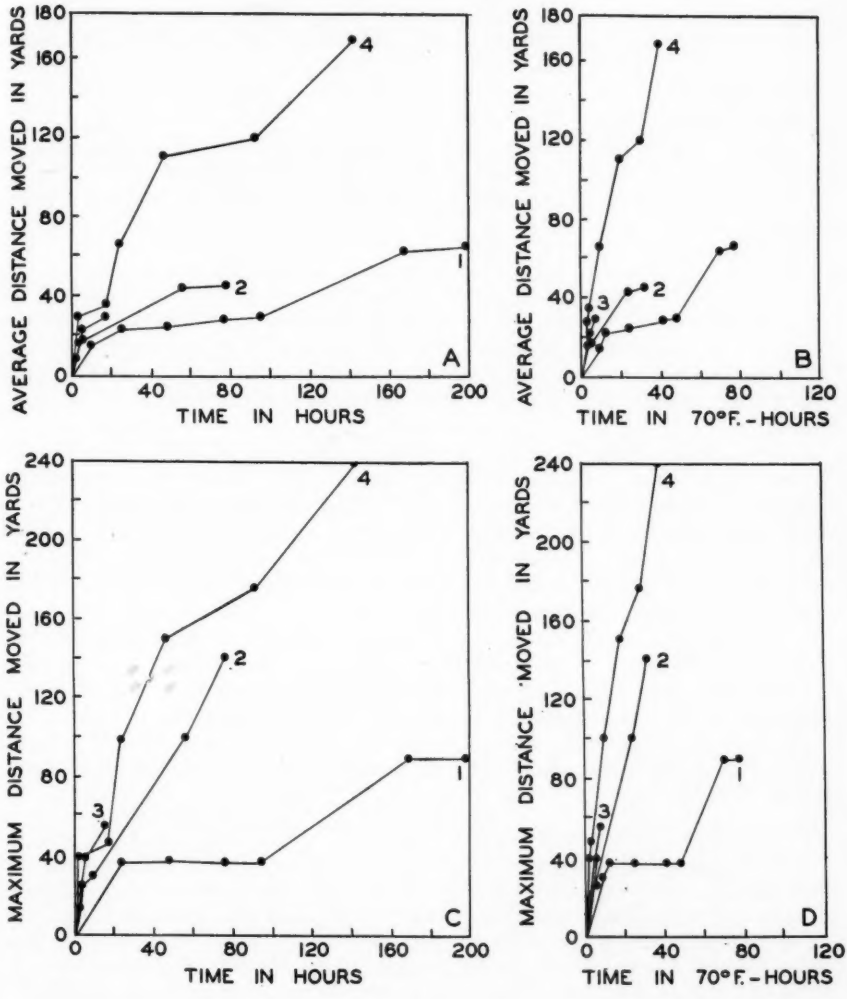

Fig. 1. Average and maximum distances of dispersal from release points by grasshoppers of different ages, tagged with P^{32} and released in non-vegetated areas. 1 and 2, *C. pellucida*, second-instar nymphs; 3, *C. pellucida*, third-, fourth-, and fifth-instar nymphs; 4, *M. m. mexicanus*, fifth-instar nymphs and adults.

threshold of significant grasshopper activity (Parker, 1930; Riegert, 1948). An hour during which the soil surface temperature, as measured by the soil thermograph, was 70°F. or more is referred to as a *70°F.-hour*.

Results and Discussion

Extent and Range of Dispersal

From what may be regarded as point sources, the grasshoppers dispersed over the following areas in the periods indicated:—

Experiment	1st	2nd	3rd	4th
Development, stage	2nd instar	2nd instar	3rd-5th instar	5th instar-adult
Area, acres	1.8	0.7	0.5	4.4
Period, days	8	3	1	6

In the periods stated, the average advance of the front of dispersal was 66 yards in the first release, 45 yards in the second, 30 in the third, and 170 in the fourth. The respective maximum distances advanced were 90, 100, 56, and 240 yards.

Rate of Dispersal

Fig. 1 shows that, as older grasshoppers were used in successive releases, the rate of dispersal increased. At the front of dispersal, second-instar nymphs of *C. pellucida* dispersed at 0.4 to 1.8 yards per hour, whereas third-, fourth-, and fifth-instar nymphs of the same species moved at 2.8 to 5.0 yards per hour. Fifth-instar nymphs and adults of *M. m. mexicanus* dispersed at 1.7 to 2.4 yards per hour.

The maximum dispersal of second-instar nymphs of *C. pellucida* within 24 hours was 37 yards (Fig. 1,C). This is almost identical with that reported by Post and Anderson (1950). Munro and Telford (1942) reported that third- to fifth-instar nymphs of *Melanoplus* sp. dispersed at an average maximum speed at 80 yards per hour. This speed is four times that reported herein. However, they do not mention the number of grasshoppers released, and the overcrowding may have produced the rapid dispersal.

On the basis of 70°F.-hours, the rates of dispersal of grasshoppers in the present investigations were three times as high as those on the basis of total elapsed time. Fig. 1,B and D, shows that second-instar nymphs of *C. pellucida* dispersed at 0.9 to 4.3 yards per 70°F.-hour, whereas third-, fourth-, and fifth-instar nymphs of the same species moved at 6.6 to 12.0 yards per 70°F.-hour. Fifth-instar nymphs and adults of *M. m. mexicanus* dispersed at 5.3 to 7.5 yards per 70°F.-hour.

Effect of Wind on Direction of Dispersal

Although age and temperature affected the activity and rate of travel of the grasshoppers, wind appeared to be the dominant factor in determining direction of dispersal. Fig. 2,A, shows the pattern of dispersal of second-instar nymphs of *C. pellucida* during successive intervals of 8 to 198 hours after release. Prevailing winds during these intervals ranged from southwest to northwest at velocities of 2 to 20 miles per hour. Most nymphs travelled farthest in southerly, easterly, and northeasterly directions from the release site; very little movement occurred against the wind except during the initial hours after release. Hence, the small grasshoppers were inclined to move with the wind.

The results of the second release (Fig. 2,B) indicated that dispersal had taken place in easterly and southeasterly directions. A relatively strong wind,

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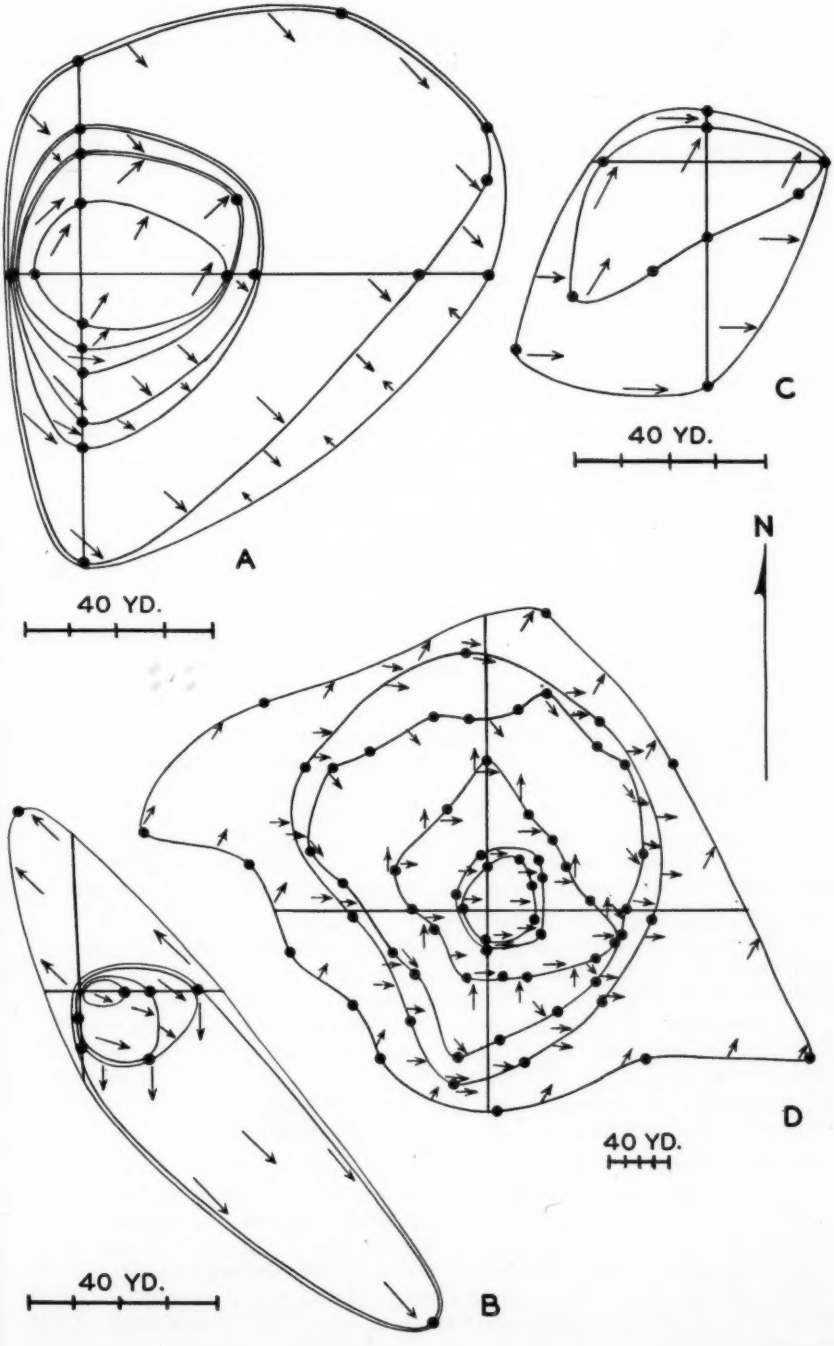
Fig. 2. Dispersal patterns of grasshoppers tagged with P^{32}. Arrows indicate the wind directions during observation periods. Lines connecting dots indicate the dispersal areas.

A, second-instar nymphs of *C. pellucida*, 8, 24, 48, 76, 97, 168, and 198 hours after release.

B, second-instar nymphs of *C. pellucida*, 1, 2, 4, 56, and 77 hours after release.

C, third-, fourth-, fifth-instar nymphs of *C. pellucida*, 3 and 16 hours after release.

D, fifth-instar nymphs and adults of *M. m. mexicanus*, 2, 17, 23, 46, 91, and 141 hours after release.


A
40 YD.
C
40 YD.
N
B
40 YD.
D
40 YD.

8 to 15 miles per hour, was blowing from the northwest during the first 56 hours after release of the grasshoppers. When the wind changed from northwest to southeast most of the grasshoppers began moving in a northwesterly direction, again downwind.

The pattern of dispersal of third-, fourth-, and fifth-instar nymphs of *C. pellucida* is shown in Fig. 2,C. In this release the nymphs proceeded south, southwest, and east from the release site. Southwesterly and westerly winds of less than 6 miles per hour prevailed. Most of the grasshoppers that moved toward the east, or with the wind, were third-instar nymphs. Those that moved toward the south and southwest, or into the wind, were predominantly fourth- and fifth-instar nymphs. Therefore, a change in direction of movement occurred relative to wind direction, the larger nymphs being inclined to move upwind and the smaller ones downwind of the release point.

The fourth field release (Fig. 2,D) produced results that corroborated observations made during the preceding three releases. The later-instar nymphs moved into the wind. Collections of radio-active grasshoppers from the periphery of the dispersal area showed that 27 fifth-instar nymphs were in the northwest quadrant, 19 in the northeast, 12 in the southeast, and 9 in the southwest. This distribution indicated the movement of more nymphs in an upwind direction from the point of release than in a downwind direction. Adults appeared to be equally distributed in all four quadrants.

The distribution of all nymphs and adults recovered seven days after the fourth release was as follows:—

Quadrant	NW	NE	SE	SW
Fifth-instar nymphs	63	62	27	39
Adults	94	85	145	33
Total	157	147	172	72

These data indicate that more adults had moved *with* the prevailing westerly and northwesterly winds than in an upwind direction. Although many adults were found in the northwest quadrant, these appeared to be recently moulted ones. This indicated that many fifth-instar nymphs had moved into the wind, moulted, and produced a relatively high population of adults in an upwind quadrant. Nevertheless, the total number of adults was greatest in the quadrant downwind of the release point, and the greatest number of nymphs were recovered in the opposite direction.

Munro and Telford (1942) observed that most later-instar nymphs headed into the wind when released. The present investigation corroborated these findings, but also showed that early-stage nymphs and adults moved downwind.

Throughout the trials, it was observed that winds exceeding a velocity of 7 to 10 miles per hour lessened grasshopper movement. On very windy days most of the activity was confined to intermittent, short crawls rather than the usual hopping movement. Many of the insects remained in small depressions or other places of shelter instead of moving about in the wind.

Dispersal Positions of Surviving Grasshoppers

In the final (fourth) experiment the number of radio-active grasshoppers recovered within 300 yards of the point of release totalled 548. No radio-active individuals were encountered farther afield. The distribution by quadrants at various distances (Table I) shows that almost 60 per cent were less than 50 yards from the release point. Only about 10 per cent had moved more than 100 yards in seven days, and the remaining 30 per cent were found 50 to 100 yards from the starting point.

TABLE I

Distribution of P^{32}– treated fifth-instar nymphs and adults of *M. m. mexicanus* Recovered Seven Days After Release in a Non-vegetated Area

Distance from release point yd.	Quadrant				Total no.	Percentage of total
	NW	NE	SE	SW		
0– 20	34	60	63	18	175	32
21– 40	31	36	52	17	136	25
41– 60	17	20	30	10	77	14
61– 80	22	11	6	7	46	8
81–100	24	5	10	11	50	9
101–140	18	8	7	9	42	8
over 141	11	7	4	0	22	4
Total	157	147	172	72	548	100

It is reasonably certain that most, if not all, grasshoppers used in these experiments had the energy resources to travel to nearby food supplies had they been able to orient themselves toward such food; yet there was no evidence that any grasshoppers moved far enough to reach green vegetation even accidentally. Their positions after seven days appeared to be the result of the forces of their own random movements plus their responses to temperature and wind direction. The net effect of movement, plus the external factors that affected it, was therefore one of diffusion rather than a wave-like or marching movement. In England, Clark (1948) observed a similar effect with colour-tagged grasshoppers released from a narrow, rectilinear site.

Effects of Overcrowding

When populations consisting of some 15,000 to 30,000 individuals were confined to caged areas of 4 to 8 square feet, they were considerably overcrowded. Fig. 1 shows that dispersal immediately after release was very rapid. The grasshoppers dispersed two to nine times as fast during the first eight hours after release as they did after one to three days.

This initial high rate of movement may be due to optical responses (Kennedy, 1945) associated with crowding. With progressive decrease in density, optical responses would become progressively less frequent. The depletion of energy from lack of food may have slowed the rate of movement, although the laboratory work of Key (1936) showed no clear trend in the rate of locomotion as the period of starvation was extended. However, Key's results do not cover nymphal stages.

Disappearance of Grasshoppers from Non-vegetated Areas

The radio-active grasshopper population liberated during the fourth field trial numbered about 15,000. The total number recovered seven days later was only 548. Therefore, about 96 per cent of the introduced population had

perished in a week's time, providing no adults had flown so far away that recovery was impossible.

Because the observations are against migration as a significant cause, losses through mortality or predation are suggested. Birds were scarce, but some predation by birds was indicated by the presence of a few radio-active bird droppings. The work of several authors, such as Washburn (1911) and Langford (1930), suggests that much of the loss in numbers was due to death from starvation.

Dead grasshoppers were not found. Observations by Putnam (1947) showed that grasshopper corpses often quickly disappear from the soil surface. Several radio-active, soil-inhabiting wireworms (Elateridae), ground beetles (Carabidae), and darkling beetles (Tenebrionidae, *Eleodes* sp.) were collected more than 20 yards from the release site. They may have picked up radio-activity by scavenging or, less probably, by predation.

Utilization of Radio-activity

Table II shows the maximum, minimum, and average counts of radio-activity for individual grasshoppers in small samples taken from treated populations before their releases, and held to permit excretion of P^{32} not assimilated. These data show that as little as 0.5 mc. of P^{32} will treat approximately 20,000 nymphal grasshoppers, notwithstanding the retention of only 10 to 18 per cent of the amount expended. Wastage of P^{32} may be attributed to initial failure of spray droplets to strike edible vegetation, and to excretion of a large percentage of the ingested P^{32}, as noted by Fuller *et al.* (in press).

TABLE II

Radio-activity Counts, in Excess of Background, of Individual Grasshopper Nymphs and Adults Tagged with P^{32} by Ingestion of Treated Wheat Seedlings

Release no.	Est. no. treated	Form tagged	Amt. P^{32} used, mc.	No. counted	Count, dis. min.			Applied radio-activity taken up %
					Highest	Lowest	Average	
1	30,000	*C. pellucida* 2nd instar	1.0	17	57,000	2,300	16,800	18.5
2	20,000	*C. pellucida* 2nd instar	0.5	18	38,500	600	8,500	17.0
3* a	10,000	*C. pellucida* 3rd, 4th, 5th instars	0.5	20	34,000	400	9,700	9.7
b	10,000	*C. pellucida* 3rd, 4th, 5th instars	0.5	14	41,500	1,150	13,400	13.4
4* a	7,500	*M. m. mexicanus* 5th instar, adult	0.5	22	55,700	1,100	21,600	15.5
b	7,500	*M. m. mexicanus* 5th instar, adult	0.5	23	57,000	700	12,000	8.9

*two cages were used for the tagging treatment but the two tagged populations were released as one.

Summary and Conclusions

1. When released on bare, cultivated fields, radio-active nymphs from the second instar and adults of *Camnula pellucida* (Scudd.) and *Melanoplus mexicanus mexicanus* (Sauss.) showed no ability to orient themselves and move toward a food supply.

2. Second-instar nymphs of *C. pellucida* moved up to 90 yards in eight days, dispersing over 1.8 acres, and moving at an average rate of 0.4 to 1.8 yards per hour, or 0.9 to 4.3 yards for every hour during which the soil surface temperature reached or exceeded 70°F. (70°F.-hour). A mixed population of third-, fourth-, and fifth-instar nymphs dispersed at an average rate of 2.8 to 5.0 yards per hour, or 6.6 to 12.0 yards per 70°F.-hour.

3. A mixed population of fifth-instar nymphs and adults of *M. m. mexicanus* moved up to 240 yards in six days, dispersing over 4.4 acres, and moving at an average rate of 1.7 to 2.4 yards per hour, or 5.3 to 7.5 yards per 70°F.-hour.

4. Early-stage grasshopper nymphs and adults moved with the wind; later-stage nymphs moved against it. Winds greater than 10 miles per hour tended to suppress activity and dispersal by keeping the grasshoppers under shelter.

5. Overcrowded populations (15,000 to 30,000 per 4 to 8 square feet) dispersed two to nine times as fast as those under uncrowded conditions. This apparently was due to increased activity caused by optical responses.

6. Seven days after release, fifth-instar nymphs and adults of *M. m. mexicanus* had not been recovered more than 300 yards from the release site; 60 per cent of the population was within 50 yards of the release point, 30 per cent had moved 51 to 100 yards, and only 10 per cent had travelled more than 100 yards.

7. Recovery of radio-active grasshoppers one week after release in a non-vegetated, well-cultivated area indicated that about 96 per cent of the population had disappeared, probably because of death by starvation and predation.

8. The radio-active isotope P^{32} was shown to be a useful tag for field studies of grasshopper movement and dispersal over a relatively short period. About 15,000 to 20,000 individuals were conveniently tagged at one time by feeding on wheat seedlings, growing on a 4-square-foot area, that had been sprayed with 50 cc. of a solution containing 0.5 mc. of P^{32}. About 14 per cent of the applied radio-activity was taken up and retained by the grasshoppers.

Acknowledgments

The P^{32} used in these investigations was obtained through Dr. J. W. T. Spinks, Head of the Department of Chemistry, University of Saskatchewan.

References

Clark, E. J. 1948. Studies in the ecology of British grasshoppers. *Trans. Roy. Ent. Soc. (London)* 99: 173-222.

Fuller, R. A., P. W. Riegert, and J. W. T. Spinks. Persistence of radio-activity in grasshoppers (Acrididae) tagged with phosphorus-32. *Canadian Ent.* 86(5): 201-203.

Kennedy, J. S. 1945. Observations on the mass migration of desert locust hoppers. *Trans. Roy. Ent. Soc. (London)* 95: 247-263.

Key, K. H. L. 1936. Rate of locomotion in relation to starvation in *Locusta migratoria migratorioides* (R. & F.) (Orthoptera). *Proc. Roy. Ent. Soc. (London)* 11: 3-6.

Langford, G. S. 1930. Some factors relating to the feeding habits of grasshoppers *Colorado Agr. Coll. Expt. Sta. Bull.* 354.

Munro, J. A., and S. Saugstad. 1938. Grasshopper migration in North Dakota. *North Dakota Agr. Expt. Sta. Bimonthly Bull.* 1: 4-5.

Munro, J. A., and H. S. Telford. 1942. Grasshopper control aided by tillage methods. *North Dakota Agr. Expt. Sta. Bull.* 309.

Parker, J. R. 1930. Some effects of temperature and moisture upon *Melanoplus mexicanus mexicanus* Saussure and *Camnula pellucida* Scudder (Orthoptera). *Montana Agr. Expt. Sta. Bull.* 223.

Post, R. L., and J. R. Anderson. 1950. Devices for marking insects. *North Dakota Agr. Expt. Sta. Bimonthly Bull.* 13: 81-82.

Putnam, L. G. 1947. Note on the disappearance of dead grasshoppers from sites of control measures. *Canadian Ent.* 79: 225-226.

Riegert, P. W. 1948. The field biology of *Camnula pellucida* (Scudd.). M.Sc. thesis. Montana State College.

Washburn, F. L. 1911. Cutworms, army worms and grasshoppers. *Univ. Minnesota Agr. Expt. Sta. Bull.* 123.

(Received February 15, 1954)

Chlorotic Spotting of Black Raspberry Induced by the Feeding of *Amphorophora rubitoxica* Knowlton[1]

By R. STACE-SMITH[2]

A chlorotic spotting of the foliage of black raspberry (*Rubus occidentalis* L.) is widespread in the coastal regions of British Columbia. The spotting first appears in June and new spots continue to develop during July and August. By mid-July, spotting occurs on virtually all plants, although its incidence varies considerably and, on some plants, only a few leaflets are affected.

In the spring of 1953, aphids were collected on various *Rubus* species in the vicinity of the University of British Columbia for raspberry virus transmission studies. Individual aphids were colonized on black raspberry seedlings in the greenhouse. Some of the seedlings developed foliar spotting identical with that noted on black raspberry plants in the field. The aphid associated with the spotting was thought to be *Amphorophora rubi* (Kaltenbach). When slides were submitted to Dr. George F. Knowlton, Utah State Agricultural College, Logan, Utah, for identification, the aphid proved to be morphologically distinct from *A. rubi* and has been described by Knowlton[3] as *Amphorophora rubitoxica* n. sp.

Experiments were undertaken to determine whether the spotting was caused by a virus transmitted by the aphid or by a toxic substance introduced into the plant while the aphid was feeding.

Relation Between Spotting and Feeding Position

The relation between the feeding position of the aphid and the symptoms was investigated. One aphid was placed on each of five leaves and its feeding position marked on a sketch of the leaf. The leaves were examined at intervals of about twenty minutes and any change in feeding position was noted. After feeding for four hours, the aphids were removed. A week later the location of the spots that had developed on the leaves was compared with the sketches.

In a few instances, no spots developed in an area where an aphid fed, but where a spot did develop, it coincided exactly with the point where an aphid had inserted its stylet into the leaf tissue. The plants were examined at intervals

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[3]Knowlton, George F. A new black raspberry aphid. Canadian Entomologist (in press).

and no further spots developed on the foliage. The spotting, therefore, does not become systemic.

This coincidence of spotting with the feeding position of the aphid supports the hypothesis that the spotting is the result of the feeding of a toxiferous aphid.

The spots appeared within four days of placing the aphids on the plants and were usually on the main or secondary veins of the leaflets. At first they were yellowish-green and about one-eighth of an inch in diameter. There was a gradual yellowing and within a week the spots became very distinctly chlorotic. If aphids were feeding on young, actively expanding leaves, growth was arrested in the chlorotic areas, resulting in a distortion and puckering of the leaf blade. A large chlorotic blotch resulting from the coalescence of several spots was common. In some instances, instead of distinct spots or blotches, leaf veinlets became chlorotic giving a diffuse net-like appearance (Fig. 1).

Fig. 1. Black raspberry leaf showing the chlorotic spotting, net-like chlorosis, and leaf distortion induced by the feeding of *Amphorophora rubitoxica* Knlt.

In addition to black raspberry, seedlings of three other *Rubus* species: namely, red raspberry (*R. idaeus* L.) Pacific blackberry (*R. vitifolius* Cham. & Sch.), and Himalaya blackberry (*R. procerus* P. J. Muell.) were tested for their response to the feeding of *A. rubitoxica.* None of these showed a positive response, although some of the Pacific blackberry seedlings showed very pale spots.

Congenital Inheritance of Spot-inducing Ability

An experiment was undertaken to determine whether the progeny of spot-inducing adults inherited the capacity to induce spots. Newly born *A. rubitoxica* were transferred to healthy black raspberry seedlings as they emerged from

viviparous adults. One young was placed on each of twenty-four seedlings and the plants were caged to prevent contamination. In every case where the newly born aphid had fed, chlorotic spotting developed on the foliage.

Since no virus is known that is inherited by the progeny of viruliferous aphids, this experiment offered further evidence that the spots are caused by the feeding of the aphid and not by a virus.

Feeding Period Required to Induce Symptoms

The feeding period required by *A. rubitoxica* to produce spots was investigated. Five aphids were transferred to black raspberry seedlings, each aphid was placed on a separate plant. After a fifteen-minute feeding period, each aphid was transferred to a second plant in the series and permitted to feed for thirty minutes, then to a third for one hour, a fourth for two hours, and a fifth for four hours. Table I gives the results of this experiment.

TABLE I

Chlorotic spotting induced on *Rubus occidentalis* by five individual aphids (*Amphorophora rubitoxica*) allowed feeding periods of varying duration.

TEST APHID NUMBER	DURATION OF FEEDING PERIOD				
	15 min.	30 min.	1 hour	2 hours	4 hours
1	−	−	+	+	+
2	−	−	+	+	+
3	−	+	+	+	+
4	+	+	+	−	+
5	−	+	+	+	+

*+ Denotes development of spots
− Denotes absence of spots.

Of the twenty-five plants exposed to aphids, eighteen developed spots. In one instance, a fifteen-minute feeding was sufficient to induce spotting, and a one-hour feeding was usually adequate.

Biological Differences Between *Amphorophora rubi* and *Amphorophora rubitoxica*

The two aphids of *A. rubi* and *A. rubitoxica* are commonly found on cultivated black raspberry plants in British Columbia. Adults of these two species are indistinguishable in the field; both are dark green to yellow green in colour and approximately three millimeters in length. These aphids are generally located at the succulent stem terminals, feeding on the stem itself or on the lower surface of the leaves.

These two species may be distinguished biologicaly by their toxemia-inducing capacity. In contrast to *A. rubitoxica*, *A. rubi* does not induce chlorotic spots on black raspberry, and no visible symptoms are observed when plants are colonized with this species.

The two species also differ in their natural host plants. Aphid colonies were collected throughout the summer of 1953 on the wild Pacific blackberry, the

cultivated black raspberry (var. Munger), and the cultivated red raspberry (vars. Cuthbert and Washington). Of the twenty-seven collections on Pacific blackberry, all were *A. rubitoxica.* On black raspberry, sixteen colonies were collected and eleven were *A. rubi* and five were *A. rubitoxica.* On red raspberry, the twenty collections made were all *A. rubi.* This indicates that Pacific blackberry is the natural host of *A. rubitoxica* and red raspberry is the natural host of *A. rubi* and that black raspberry is a mutual host of the two species.

Another biological difference between these two species became evident when their ability to transmit raspberry viruses was investigated. Twenty colonies of *A. rubi* and forty-eight colonies of *A. rubitoxica* were tested for their capacity to transmit two raspberry viruses, leaf mottle virus and yellow mosaic virus. Both viruses were transmitted with every colony of *A. rubi* whereas neither virus was transmitted by *A. rubitoxica.*

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A New Black Raspberry Aphid

By GEORGE F. KNOWLTON[1]
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An apparently undescribed *Amphorophora* was received from Richard Stace-Smith of the Canada Agriculture Plant Pathology Laboratory, Vancouver, B.C., Canada. This species produced leaf symptoms on Munger black raspberry, *Rubus occidentalis*, which differed from anything associated with the feeding of *Amphorophora rubi* (Kaltenbach). An examination was made of the material by Professor M. A. Palmer, Dr. F. C. Hottes and the writer. This failed to place this blackberry aphid as a described species. Therefore, it is here described as new.

Amphorophora rubitoxica n. sp.

Alate vivipara: Color most commonly dark green, but a few were yellowish green; body 2.3 to 2.85 mm. long and 1.42 across the abdomen; width through eyes .5; ocular tubercles present; frontal tubercles well developed; antennae blackish to black, usually darker beyond base of III; antennal III, .88 to 1.03 mm. long, with 26 to 33 circular sensoria along most of antennal length, arranged largely in a row; antennal IV, .55 to .88; V, .54 to .81; VI, .121 to .173 plus .91 to 1.21 mm. long; rostrum reaches between 3rd coxae; rostral IV plus V blackish to black, .19 to .2, tip slenderly obtuse; wings with prominent, dusky veins; hind tibiae 2.28 to 2.73 long, dusky to black on distal enlarged area; hind tarsi .13 to .138; cornicles dusky to largely black, .60 to .77 mm. long, appearing largely smooth, but with cuticula of narrower basal 2/5 being minutely wrinkled, and with light imbrications toward distal end; swollen area of cornicle nearly twice the diameter of cylindrical proximal portion; cauda pale, total length .3 to .45 mm. long, with 2-pairs of lateral hairs and two dorsal hairs on distal one-half of surface; anal plate pale, broadly rounded (to somewhat flattened across end).

Type: Collected on *Rubus vitifolius* Cham. and Sch. at Vancouver, B.C., Canada, September 22, 1953 (R. Stace-Smith). *Paratypes* same host and locality, September 15, 1953 (R. Stace-Smith). Type slide deposited in the Canadian National Collection; paratype in the collection of the writer.

Apterous vivipara: Color dark green, a few being yellowish green, body pale in cleared specimens; body 2.9 to 3.24 mm. long and 1.38 to 1.58 wide across

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abdomen; width through eyes .59; antennal tubercles prominent; antennae dusky to blackish; antennal III, 1.03 to 1.12 mm. long, with 9 to 12 circular sensoria on proximal one-half of segment; antennal IV, .74 to .78, without sensoria; V, .55 to .56; VI, .138 plus 1.05 mm.; rostrum reaching between or slightly exceeding 3rd coxae; rostral IV plus V dusky to blackish, slenderly obtuse, .19; hind tibiae 1.85 to 2.66 mm. long, dusky to blackish, darkest on distal area; hind tarsi .137 to .144; cornicles similar to alates, .81 to .88 mm. long, darkest over swollen area comprising distal three-fifths of cornicle length; cauda pale, .38 to .55 mm. total length.

Paratypes: On *Rubus vitifolius* at Vancouver, B.C., Canada, September 15, 1953, August 17, 1952, and August 23, 1953 (R. Stace-Smith). Reportedly collected also at Abbotsford and Saanichton, but these specimens were not examined.

Taxonomy: *Amphorophora rubitoxica* runs to *A. tigwatensa* Hottes, in Knowlton and Allen's key (Canadian Ent. 77:111-112, 1945), from which it differs in having shorter and more swollen cornicles and shorter antennal joints III to VI. It differs from *A. rubi* in having shorter antennal III with fewer sensoria on it, shorter cornicles and longer rostral IV plus V. From *A. agathonica* Hottes it varies in much shorter antennal segments and cornicles, and fewer antennal sensoria. It differs from *A. utahensia* K.-A. in having darker antennae, shorter antennal III with fewer sensoria, and shorter and darker cornicles. It differs from *A. rubicumberlandi* K.-A. in having a similar length antennal III but fewer sensoria in the alate forms, and in possessing a longer rostral IV plus V, long cornicles and cauda.

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Note on a Staphylinid (Coleoptera) Predator of Earthworms

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In August, 1953, the proprietor of an earthworm-propagating establishment in Vancouver, British Columbia, reported that her propagating boxes were infested with small, dark-brown beetles. Specimens of the beetles were identified by Professor M. H. Hatch, University of Washington, as of *Quedius* (*Microsaurus*) *mesomelinus* (Marsh.), Coleoptera, Staphylinidae.

During laboratory feeding tests, both larvae and adults of *Q. mesomelinus* killed and fed on immature and mature forms of *Eisenia foetidus* (Savigny) but not on the eggs in the cocoons. The young earthworms were completely devoured but the larger, more mature individuals were only partly devoured. The staphylinids fed only on freshly killed hosts.

(Received March 23, 1954)

A Species of *Cephalosporium* (Moniliaceae) Causing a Fungous Disease in Larvae of the European Corn Borer, *Pyrausta nubilalis* (Hbn.) (Lepidoptera: Pyraustidae)[1]

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During the summer of 1951, a number of full-grown larvae of the European corn borer, *Pyrausta nubilalis* (Hbn.), that died while being held in cold storage for experimental use at the Entomology Laboratory, Chatham, Ontario, were forwarded to the Kingston laboratory for diagnosis. Microscopic examination showed that the dead larvae contained a hard core of fungous mycelium.

The Causative Fungus

Isolations of fungi from the dead larvae were made by plating samples of the hard core on Littman oxgall agar (Difco) (Littman, 1947) and stock cultures were grown and maintained on slants of Sabouraud maltose agar (Difco). Study of slide cultures, by the method described by Littman (1949), showed that the fungi from all larvae sampled were of the same species.

The fungus was identified by D. M. MacLeod, Insect Pathology Laboratory, Sault Ste. Marie, Ontario, and J. W. Groves, Botany and Plant Pathology Division, Ottawa, as a species of *Cephalosporium*. This disease is of special interest as members of this genus are commonly parasitic on scale insects and there is no previous record of any species of this genus infecting larvae of the European corn borer.

The characteristics of the fungus when grown on Sabouraud maltose agar in slide culture are as follows: mycelium cottony and hyaline; hyphae elongate, separate, septate, and branched; conidiophores mostly verticillate (Fig. 1); conidia distinct from the conidiophores, globose, hyaline, one-celled, single or in a loose head at the tips of the conidiophores, size 1.3–1.9 microns x 2.8–4.5 microns.

Cephalosporium sp. grew readily on artificial media and developed particularly well on potato infusion agar (Difco). The conidia germinated as well in sterile distilled water as in the presence of nutrient substances. In Bacto-nutrient broth (Difco), at 26°C., germination commenced in 20 hours and some mycelium was evident after 24 hours of incubation: there was luxuriant growth after 40 hours. On potato infusion agar, the rate of growth was studied at six temperatures ranging from 20.5°C. to 35°C. The most rapid and luxuriant growth was observed at 26°C.; there was slow growth at 20.5°C. and 29°C. and no growth at 35°C. In Sabouraud maltose broth, the surface growth of the fungus was directly proportional to the amount of maltose in the medium, between the limits of 5 and 40 gm. of maltose per litre of medium.

Symptoms and Pathology

The symptoms of infection in corn borer larvae were noted after injection of spore suspensions in distilled water. At first, reddish-brown spots appear in the integument. Then the entire larval surface darkens slightly. The spots increase in number and the darkening process continues so that at death the entire surface of the integument is dark brown. Meanwhile there is a gradual decrease in

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activity and reaction to touch. The last symptom to appear before death is a general softening of the body; this persists after death until the mycelium fills the body and the characteristic hardening occurs. At a high relative humidity the hyphae issue through the integument and white cottony mycelium finally covers the external surface of the larva. Conidia are produced by the external mycelium 24 to 48 hours after death.

When larvae were allowed to crawl over growing cultures of the fungus, mycelial growth developed rather rapidly on the integument but penetration was slow and the larvae died before there was any visible fungous growth in the body cavity. Death occurred in 48 to 72 hours and the external symptoms of infection were the same as for larvae injected with a spore suspension.

The growth of the fungus and its effect on the larvae during development were determined by study of histological sections of normal and infected larvae incubated at 26°C. Larvae were fixed in Kahle's acid fixative for 24 hours at 60°C., washed, and stored in 70 per cent alcohol. They were dehydrated in dioxane, embedded in paraffin, and sectioned at a thickness of 10 microns. Larvae injected with a water suspension of 1.08×10^6 spores were fixed for sectioning at hourly intervals.

Twenty hours after injection, spores were found singly and in small concentrations in the body cavity and, although somewhat swollen, none had produced germ tubes (Fig. 2). When the first external symptoms of infection were visible, 24 hours after injection, there were small concentrations of mycelium in the blood lacunae under the integument (Fig. 3). At death, 31 hours after injection, a few concentrations of mycelium were observed in the body fluid and among the fat cells but there was no apparent breakdown, lysis, or other cytological change in the cells or the tissues. After death there was a progressive growth of the fungus in the body cavity. Twenty-four hours after death the mycelium filled the body cavity (Fig. 4), the fat cells had broken down, and hyphae had started to penetrate the integument.

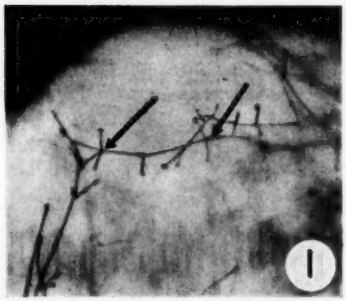
Larvae infected by external contact were fixed for sectioning only at the time of death. From the external concentrations of mycelial growth, hyphae had penetrated through the cuticle (Fig. 5) and in a few cases through the hypodermis, but there was no mycelial growth evident in the body cavity. In some invaded areas of the integument there was a pronounced disintegration of the outer portion of the cuticle, suggesting a chemical action.

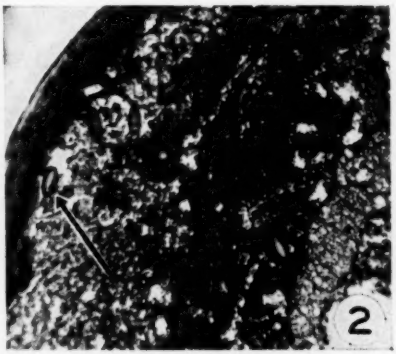
By either route of infection, there was no extensive mycelial growth in the body cavity of the larvae at the time of death.

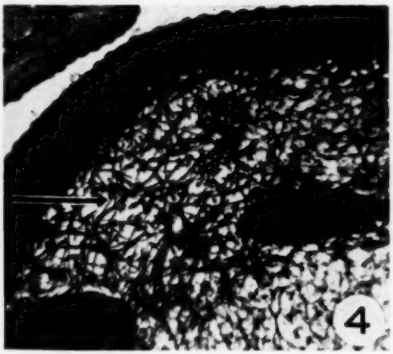
Pathogenicity Tests

Pathogenicity tests were made with mature corn borer larvae in which there was no evidence of any disease. All treated larvae were incubated at 26°C. in glass vials containing moistened filter paper, which maintained the relative humidity very close to 100 per cent.

External infection occurred when larvae were dipped in sterile water and were rolled or allowed to crawl over a growing culture of the fungus. When larvae were left on the culture for one hour, 40 per cent became infected and died; when left for two hours, 70 per cent died. These figures are the averages of two tests, 10 larvae being used for each test. To demonstrate that death was not due to ingestion of spores, the oral openings of ten larvae were sealed with wax before they were placed on the culture. All of the larvae became infected; six died within 48 hours, and the remaining four during the next 24 hours.







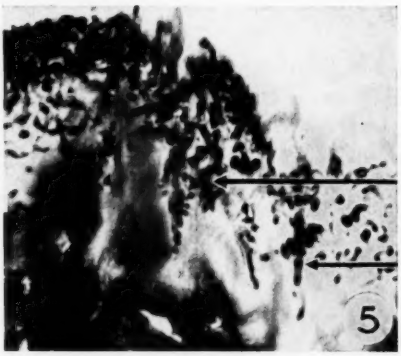


Fig. 1. Verticillate branching of conidiophores of *Cephalosporium* sp. (x 125)

Fig. 2. Part of a longitudinal section of a larva of the European corn borer 20 hours after injection, showing position of the spores. (x 300)

Fig. 3. Extent of development of the fungus in an injected larva when the first symptoms of infection were visible. (x 300)

Fig. 4. Extensive mycelial growth in an injected larva 24 hours afer death. (x 300)

Fig. 5. Part of a longitudinal section through a larva infected by the eternal application of the fungus, showing penetration of the cuticle by numerous hyphae. (x 700)

Injection of larvae was tested with spores in water suspension at various concentrations. Tests in which 10 larvae were injected with 0.01 ml. containing 50, 500, 5,000, 50,000, or 500,000 spores gave the following results: at doses of 50 and 500 spores, the mortality was zero; at 5,000 spores, 70 per cent; and at 50,000 and 500,000 spores, 100 per cent. Thus the LD_{50} lies between 500 and 5,000 spores per larva. The average time of death varied from 29.0 hours when 500,000 spores were injected to 77.0 hours when 5,000 spores were injected.

No deaths occurred when injections were made of similar numbers of heat-killed spores of *Cephalosporium* sp. or of viable spores of a species of *Penicillium* isolated from larvae of the larch sawfly, *Pristophora erichsonii* (Htg.). This indicated that the introduction of a large number of foreign bodies did not cause the death of the corn borer larvae.

Summary

A species of *Cephalosporium* was isolated as the causal agent of death in a collection of mature larvae of the European corn borer being held in storage. Larvae were readily infected by contact with growing cultures of the fungus, and injection of 0.01 ml. of heavy spore suspensions. There was no extensive mycelial growth by either route of infection at the time of death of the larvae.

Acknowledgment

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References

Littman, M. L. 1947. A culture medium for the primary isolation of fungi. *Science* 100 (2744): 109-111.

Littman, M. L. 1949. A special microslide for the culture of fungi. *Am. J. Clin. Path.* 19: 278-283.

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